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Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 9

- Characterising novel anti-schistosomals

  Schistosoma mansoni, helminth, vaccine, drug

- Oscillatory brain activity and perception

  Sensory perception, brain rhythms

- Genetics of cardiovascular development

  Heart development, congenital heart disease, genetics

- Testing products and methods for aquaculture

  Aquaculture, veterinary medicines

- Effects of nutritional components on health and ageing

  Diet, immune response, aging, health

- Developmental and Reproduction Safety Testing Using Small Animal Species

  Reproduction Safety Assessment Small Animal

- Developmental Regulation of Physiological Systems

  Fetus, Pregnancy, Developmental physiology, Early life programming

- Safety Testing of Medicinal Products Using Non-Human Primates

  Regulatory Safety Assessment, Non-Human Primates

- Avian Ecotoxicology studies

  Avian, environmental, risk assessment

- Identifying interventional targets – kidney disease

  Kidney fibrosis, nephropathy

Characterising novel anti-schistosomals
Schistosoma mansoni, helminth, vaccine, drug

- Summarise your project (1-2 sentences)

The overall goal of our research programme is to identify novel vaccine, chemotherapeutic and immunomodulatory agents useful in combating the neglected tropical disease schistosomiasis. By applying *in vitro*, *in vivo* and *ex vivo* models, we will characterise how selected schistosome biomolecules affect parasite development, mammalian cell phenotypes and host/parasite interactions.

- 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 pursuing our research programme because: 1) there currently does not exist a suitable anti-schistosome vaccine and 2) existing control strategies rely on the effectiveness of a single drug, which has an unknown mechanism of action and is incapable of preventing reinfection in endemic areas. The identification and characterisation of immunomodulatory biomolecules also provides information relative to the mechanisms schistosomes use to orchestrate long-term survival in infected hosts. This information could be used to direct more effective immune responses during our search for novel anti-schistosomal drugs or vaccines.

- Outline the general project plan.

The causative agent responsible for schistosomiasis, *Schistosoma*, cannot develop normally outside a suitable vertebrate host. Animal models are, therefore, absolutely essential in achieving our goal of identifying viable vaccine, chemotherapeutic and immunomodulatory agents. The primary use of animals will be to maintain the complete *Schistosoma* lifecycle (snails as intermediate hosts and mice as definitive hosts) in order to access parasite material for *in vitro* studies. Secondary uses of mice will be for *in vivo* testing of prioritized novel chemotherapies and vaccines, once suitable candidates are properly characterized from the *in vitro* studies.

- 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.

Protocol (1) involves the percutaneous or intraperitoneal administration of *S. mansoni* larvae (obtained from snails) to mice (~1000 mice). In order to generate schistosome parasites of different lifecycle stages for our studies, these organisms must develop and mature in a suitable definitive mammalian host. The expected adverse effect of this protocol will be stress due to handling, irritation due to parasite infection and deterioration of condition due to parasite development. Collectively, we would classify these adverse effects under a moderate severity limit.

Protocol (2) will allow us to assess the immunoprophylactic potential of characterised schistosome biomolecules (~350 animals). Here, we will administer protein or DNA vaccines (intraperitoneal, subcutaneous/intradermal or intramuscular injections) to mice before percutaneously infecting them with schistosomes. At seven weeks after infection, we will assess the efficacy of vaccination when compared to control animals. The expected adverse effect of this protocol will involve stress due to handling, mild discomfort of vaccination/muscle electroporation, irritation due to parasite infection and

deterioration of condition due to parasite development. Collectively, we would classify these adverse effects under a moderate severity limit.

Protocol (3) allows us to examine the function of schistosome biomolecules (~350 animals). By using RNA interference (RNAi) or targeted drug treatment, we will be able to generate information critical to our understanding of proteins necessary for intra-mammalian parasite development. Here, *in vitro* manipulated (using RNAi) parasites will be injected intraperitoneally into mice or infected mice will be treated (intraperitoneally, transdermally or orally) with an anti-schistosomal chemotherapeutic agent. Both of these *in vivo* manipulations can synergistically be used to assess the importance of key schistosome biomolecules. The expected adverse effects of this protocol include stress due to handling, irritation due to chemotherapy administration or parasite infection and deterioration of condition due to parasite development. Collectively, we would classify these adverse effects under a moderate severity limit.

Protocol (4) facilitates the production of antisera or immune cells necessary for the study of schistosome biomolecules (~100 mice). In one use of this protocol, schistosome proteins (or DNA constructs encoding schistosome proteins) will be administered intraperitoneally, intradermally or intramuscularly into recipient mice. Antisera will be collected from immunised mice to use in downstream *in vitro* assays. In a second use of this protocol, a macrophage-inducing reagent will be administered intraperitoneally into recipient mice. Immune cells will be collected from these mice (or Schedule 1 killed mice) to use during *in vitro* assays. The expected adverse effects of this protocol include stress due to handling as well as irritation due to intraperitoneal, intradermal or intramuscular injections. Collectively, we would classify these adverse effects under a moderate severity limit.

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

Our search for new drug targets, immunomodulatory agents and vaccine candidates is an important priority for developing novel strategies to combat this major human pathogen. It is anticipated that this research agenda will reveal key parasite molecules critical in establishing long-term host relationships. These molecules will be the subject of peer-reviewed manuscripts, presentations at scientific meetings, grant applications and follow on work by the scientific community.

- 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.

We anticipate using 1800 mice in total throughout the 5-year licence. This model is the most easily adaptable and appropriate vertebrate definitive host for studying schistosomiasis in the laboratory as it is a fully permissive host, supports the full sexual development of the parasite and produces fully viable, infective larval stages. In addition, the mouse model can easily be genetically manipulated with a wide range of knockout and transgenic lines available. This allows for specific dissection of particular host factors that may or may not be involved in the proposed experimental procedures discussed herein. Finally, the mouse is the most highly tested animal model system (lowest vertebrate group) for determining levels of vaccine-induced protection or effects of chemotherapeutic treatment in our field.

To minimise the number of mice necessary to obtain statistically relevant results, power calculations for determining optimal group sizes will be derived, inbred mice will be used and careful planning of all experiments will be performed in consultation with IBERS

statisticians.

- 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.

Schistosomes are mammalian parasites and, thus, necessitate a suitable mammalian host for completing studies outlined in our research programme. While we can employ other non-mouse alternatives for some study objectives, this is not possible for *in vivo* determination of novel chemotherapies, vaccines or immunomodulatory agents.

Non-mouse alternatives include *in vitro* parasite culturing manipulations, appropriate *in vitro* and *ex vivo* cell model surrogates to assess how a schistosome biomolecule can affect a mammalian cell, molecular biology manipulation of schistosome genes, comparative genomics/bioinformatics to computationally interrogate schistosome biomolecules, functional genomics to suppress schistosome gene expression, epigenetics to assess schistosome development, biochemistry to assess enzyme activity and proteomics to understand the biological nature of key schistosome molecules. All of these non-mouse alternatives will be synergistically used to prioritise schistosome biomolecules prior to performing *in vivo* experiments in the mouse model of schistosomiasis.

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

All animal protocols employed in this license have been carefully developed over the last several decades in laboratories around the world and have been the subject of peer review. Our specific experiences utilizing animal models for the study of schistosome/host relationships have come about from 18 years of practical experimentation. All procedures use animals obtained from a licensed supplier and all animals are housed in conditions that comply with the Animals Directive Code of Practice (2010/63/EU).

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Oscillatory brain activity and perception</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Sensory perception, brain rhythms,</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)[1]</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[2]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project aims to understand how sensory precepts arise from the brain’s neural activity. We study how slow oscillatory activity shapes how sensory information is processed and determines which sensory objects are perceived. Oscillatory activity is present in many brain structures but its causal impact on how neural networks represent the sensory environment and influence perception is little understood. The objectives of this project are to perform both correlational and causal tests to elucidate the impact of slow brain rhythms on information encoding and perception.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This work provides an improved understanding of the physiological mechanism underlying perception and bridges between the microscopic level of individual neurons and the macroscopic scale accessible in non-invasive human studies. The specific insights generated here may be later translated to human studies and may be of great relevance for understanding brain dysfunction and clinical states associated with changes in oscillatory activity. This project hence provides critical knowledge that may facilitate the development of sensory prosthesis (e.g. cochlea implants) and may contribute to a better understanding of the role of slow oscillatory in clinical conditions that have previously been linked to changes in oscillatory brain activity (e.g. Schizophrenia).</td></tr>
<tr><td>What species and approximate numbers of</td><td>This project uses rodents (mostly rats) and the number of animals will be around hundred and thirty over five years.</td></tr>
</table>

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

animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All invasive procedures are carried out under general anaesthesia, analgesia, using aseptic conditions and appropriate post-operative care. Potential adverse effects may be *discomfort* relating to surgical induction and recovery or relating to the implant. The expected severity is moderate. *In very unlikely case of major post-surgical or other unexpected suffering and at the end of the procedures the animal will be killed humanely.*
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	An animal model is needed because we require localized intracranial measures of neural activity simultaneously with behavioural readings. Such local measurements of neural activity are currently not possible in humans, and ex vivo preparations preclude behavioural readings. However, the results obtained here may constrain the interpretation of non-invasive methodologies (e.g. EEG/MEG) such that these may be better utilized in non-invasive studies in the future and replace some of the protocols currently requiring animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experimental design and methods of analysis have been discussed with expert statisticians, veterinary advice, and follow the current state of the art for this kind of research. The study is designed such that a single animal contributes a large amount of data, and we use advanced analytical methods and computer simulations to make use of this data for several research questions.
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 smallest animal species (rodents) suitable for these questions has been chosen and all procedures are well established for this. Rodents can be trained on sensory tasks and display many cognitive and sensory capabilities that are principally similar to humans. Smaller and less develop mammalian models with sufficient knowledge about brain organization and the necessary behavioural protocols do not exist. Animal wellbeing is continuously monitored by the general state of health (incl. weight and feeding), spontaneous behaviours, and clinical and physiological parameters.

Genetics of cardiovascular development
Heart development, congenital heart disease, genetics

- Summarise your project (1-2 sentences)

This project will use genetically modified transgenic mouse models to uncover genetic pathways that control cardiovascular development. We will focus on genes that are responsible for activating other genes (known as transcription factors), that when mutated result in congenital heart disease in the developing mouse. We will also investigate the molecular and cellular process involved in building the aberrantly developed heart and its associated blood vessels, as well as employing cell culture based assays.

- 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.

Congenital heart disease is a major cause of morbidity and death in childhood in the West, with genes playing a major role. However, the genetic and developmental mechanisms underlying most congenital heart disease remain unknown. Some patients with congenital heart disease have syndromes where certain genes have become mutated or are missing but it is thought that the syndrome can be further affected by mutations in other as yet unknown genes. This research aims to identify genes that work together in a network to control normal cardiovascular development.

- Outline the general project plan.

In this project we will:

1. Investigate the genetic pathways that control normal cardiovascular development
2. Use transgenic mouse models to manipulate gene expression and examine the effect of gene loss or mutation on cardiovascular development
3. Investigate the cellular mechanisms that contribute to cardiovascular development
4. Investigate the molecular mechanisms that control gene expression in relation to cardiovascular development

- 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 majority of mice used in the project licence will need to be identified by examining the mouse's DNA, and material for this will be obtained by taking a piece of tissue from the ear. This procedure is quick and should only cause mild and short-lived pain. As we will mainly be examining embryos or foetuses taken from pregnant females, the procedure of mating transgenic mice will be a normal and natural act that should have no adverse effects. In some cases we will need to administer substances by injection or oral routes, but we do not expect these to cause any adverse effects. All mice will be closely monitored following the administration of substances to ensure that no adverse effects occur. Very few adult transgenic mice will have clinically adverse effects as harmful effects will occur in the embryo before it is two-thirds of the way through gestation. These effects will result in some mice dying shortly after birth, mainly from heart defects.

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

Congenital heart disease affects approximately 1% of all births and is a major burden for the patient as well as the health care system. By furthering our understanding of the genetic pathways that control cardiovascular development we may be able to devise screening strategies for prospective parents that can highlight any potential risk to the child.

- 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.

We will breed up to 100 mice of each strain listed on the project licence per annum, of which 90% will be used for intercrossing with other strains. Similar numbers of the intercrosses will also be maintained. Embryos (which are less than two-thirds through the gestation period) or foetuses (which are greater than two-thirds through the gestation period) will be collected from timed matings for analysis. We will not exceed the 10,100 adult mice, 500 neonates and 9,000 foetuses over the 5 year duration requested on the project licence. Mice are being used in this project because this is the simplest organism that has a similar heart and blood vessels to human, and that can be used to investigate the roles of different genes in cardiovascular development. In the majority of cases we use embryos or foetuses for analysis. All experiments are designed to use the least numbers of animals. We regularly consult a statistician for advice so that experiments are properly designed and group sizes are the minimum number required to give an accurate answer. We also use a magnetic resonance imaging technique which allows for foetuses to be imaged without destruction, therefore the foetuses can be reused for analysis with other techniques. Moreover, the datasets generated of the foetuses can be shared with other researchers. Our use of *in vitro* culturing of embryos will also reduce the overall number of animals used since the unit of experimentation can be the single embryo, and not the entire litter.

- 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.

Unfortunately, due to the complexity of the processes involved in cardiovascular development, there are no suitable cell culture systems that can be used to replace mouse models. Mice are being used in this project because this is the simplest organism that has a similar heart and blood vessels to human, and that can be used to investigate the roles of different genes in cardiovascular development. In the majority of cases we use embryos or foetuses for analysis or, if appropriate (for example when investigating processes that occur within individual cells), then cell culture experiments are used. Cells grown in culture are a useful tool to investigate how different proteins interact or how genes can be controlled (i.e. switched on and off) by other genes. These sorts of experiments will be conducted in parallel with the mouse studies to identify molecular and biochemical mechanisms that control the genes we are studying.

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

Most of our mice are used for maintaining genetic lines and have no abnormalities. As we are investigating the effect of genetic mutations on the developing embryo or foetus, the main procedure we carry out is by inter-breeding different transgenic strains so that embryos or foetuses can be collected from the pregnant dam. Because mating is

considered a natural act, this does not result in any abnormal suffering for the mice.

Testing products and methods for aquaculture
Aquaculture, veterinary medicines,

- Summarise your project (1-2 sentences)

We use fish to test potential new medicines and treatments that may be used to combat diseases and parasites. These may be drugs, vaccines or wrasse used as “cleaner fish”.

- 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 aquaculture there are diseases for which no cure exists, parasites that are developing tolerance of available medicines, and new problems arising with the expansion of the industry. We develop new treatments such as medicines, vaccines or biological controls as a service that also allows sensible combinations for drugs for new farm sites and integrated management.

  We study fish health on a small scale under controlled conditions that allow us to measure the effects of new husbandry methods or disease treatments. The work is needed in order to keep farmed fish healthy so that they don’t suffer and to ensure that the methods employed are efficient and ecologically sound.

- Outline the general project plan.

80% of our research is for commercial clients in the feed, aquaculture and pharmaceutical sectors. Projects are usually a sequence of steps in identification, testing, safety assessment and approval of new medicines. Towards the end of the development process for a new product work to Good Laboratory Practice (GLP) guidelines.
Other academic work is for the Institute of Aquaculture at University of Stirling, which can include making fish feeds more environmentally sustainable, developing vaccines, or growing cleaner fish.

- 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.

  Whenever we are testing efficacy, or effectiveness, of a medicine we have to give a disease or parasite which may cause harm. When we test a potential medicine for safety to the target animal we give several times the expected therapeutic dose, which can cause toxic effects that depend on the type of drug, or even death.

  If present in large numbers external parasites can cause irritation to the skin or gills that may lead to the formation of lesions or mortality. External bacterial or fungal infections can also lead to external lesions or mortality. Systemic bacterial or fungal infections can lead to internal lesions and mortality.

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

The aquaculture industry is an increasingly important source of protein for the diet of the world’s population, and as more aquatic species are domesticated for intensive farming a limiting factor will be keeping up with the range of infections. In salmon aquaculture for example sea lice are controlled but are beginning to show reduced sensitivity to current medicines, while amoebic gill disease can only be controlled with fresh water or hydrogen peroxide in large volumes.
The service we provide allows timely development of new treatments for such infections that will preserve the welfare of many millions of fish which will then provide food for millions of people.

- 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.

Most of the animals we use are Atlantic salmon to developing treatments to support the salmon aquaculture industry. Wrasse are an ecologically-friendly cleaner fish in salmon farms, and since we share a location with a commercial farm we help them improve rearing, feed, husbandry and use.

We use small-scale efficacy tests to determine effect size and inform power calculations to estimate the numbers required for a significant result. We then plan experimental protocols to include the minimum number of animals that will guarantee a valid result taking into account a likely level of withdrawals and mortalities from coincident disease, handling injuries or other sources.

- 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 are testing treatments for pathogens and parasites that can only reproduce and grow on their live animal host, such as sea lice that grow on salmon. Therefore we have to use live marine fish at least as the hosts to establish a supply of the pathogen or parasite, even where it is possible to test the new treatments in vitro away from the fish.

  Our work is aimed at identifying and demonstrating the safety of methods for treating fish in large cages or ponds, either in freshwater or at sea. In these contexts the delivery of a treatment is usually either by incorporation into feed, exposure in a bath, or injection, and to obtain approval we have to test treatments using whichever of these is first choice for use in farms.

  Where it is possible to culture a pathogen or parasite in vitro for even a part of its life cycle or to test in vitro we do so, such as when hatching sea lice eggs or quantifying drug sensitivity of adult sea lice.

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

  Many of the treatments that we test for pharmaceutical clients are already known to be highly selective for the target disease and safe in fish because they are licensed for use in agriculture, which minimises the risk of adverse reactions.

  We often carry out range-finding tests in vitro to establish the effective dose for a new drug before using it in vivo.

  We carry out small scale toxicity safety tests as the first live phase in order to avoid exposing large numbers of animals to harmful doses.

  When designing a live phase of a project we can usually choose a relatively low number of animals. This is possible because we are assessing whether a treatment has a strong therapeutic effect that is easily demonstrated statistically. Looking for large effects also means that we can set the level of parasite challenge low and demonstrate removal rather using more to induce pathology, such that even many untreated animals will never suffer.

  We monitor the health of all the animals we use every day to ensure that we cause the minimum of suffering. This is written into every contract and protocol in order to ensure that we always have the resources available. If an animal is found to be suffering it will always be withdrawn from the study and humanely killed.

Effects of nutritional components on health and ageing
Diet, immune response, aging, health

- Summarise your project (1-2 sentences)

This project investigates how diet can affect many aspects of health and in particular modulate the severity of chronic inflammatory conditions.

- 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.

Diet affects the functioning of several systems, including liver, kidney and cardiovascular function, and immune responses. The effects of fatty acids on immune responses are facilitated by the anatomical relationship between lymphoid and adipose tissues. Lymph nodes, where immune responses take place, are embedded in fat, and the response mounted by a node to an immune challenge is provisioned by adipocytes surrounding it. Upon receiving signals from an immune-challenged lymph node, adipocytes respond by releasing fatty acids, derived from the diet, that are used locally by cells within the node as a source of both energy and building blocks to mount the response. We hypothesise that the availability of precursors for lipid-based regulatory molecules varies with diet.
The questions we ask are:

1. How does diet quantitatively affect biomarkers of health over time?
2. How do dietary fatty acids quantitatively affect the peripheral inflammatory immune response?
3. What is the mechanism of delivery of dietary fatty acids to the site of the immune response?
4. Do immune responses in the gut resemble mechanistically those in the periphery, and are they also modulated by dietary components?
5. How well can our 3D co-culture model reflect the *in vivo* interactions between diet and immune response?

- Outline the general project plan.

Rats are fed from weaning on one of a number of experimental diets. They are maintained under normal husbandry conditions, with an enriched environment. The rats all grow at a normal rate and achieve the same range of body masses. After times ranging from one day to 18 months eating the diet, the rats are harvested. Some are subjected to an immune challenge, given orally or by injection. The immune challenges are transient and low level. At various times following the immune challenge, the rats are humanely killed and their tissues analysed for evidence of the progress of the immune response and for other markers of health.

- 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 most likely adverse effect of the procedures is minor bruising around the site of administration of the immunogen, but we have never observed any signs of distress in treated animals.

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

The project provides a deeper understanding of the mechanisms by which particular foodstuffs can affect health, and how immune stimulants deliver their effects. It can inform dietary choices, particularly for individuals who are immune-compromised, or who have pre-existing chronic inflammatory conditions. The animal experiments are vital because only by using complex organisms can the layers of interaction between different body

systems be studied.

- 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.

We take advice from a statistician to ensure that we use the minimum number of rats consistent with robust results. We use a maximum of 200 rats per year (total 1000 over the project life), but this number is likely to fall as we have developed a tissue culture system that can yield data at a molecular level for some of our experiments (see below).

- 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 animals in our experiments because there is currently no experimental system that mimics the complex physiological interactions that we study. We use rats because they are mammals and have physiological responses similar to those in humans; furthermore they are not fussy eaters and thrive on the variety of diets that we use. We have developed a tissue culture system that has reduced our animal use substantially, although it cannot replace them altogether.

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

The adverse effects of our experiments are few, with the most likely being bruising around the site of administration of immune stimulant. This is minimised by careful animal handling by experienced staff.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Developmental and Reproduction Safety Testing Using Small Animal Species</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Reproduction Safety Assessment Small Animal</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td colspan="3"><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>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[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 colspan="3">Humans are exposed to xenobiotic materials as patients, consumers and workers. In order to allow sound regulatory decisions regarding safe human exposure levels to xenobiotics, it is essential to conduct a risk assessment by relating the intrinsic hazard profile of the material to the desired or likely exposure in man.
This project licence authorises the conduct of in-vivo studies in laboratory small animal species to evaluate the hazard profile of xenobiotics in terms of developmental and reproduction toxicity, and toxicokinetics.</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">The principal benefit of this project is the provision of safety data to facilitate sound regulatory decisions regarding human exposure to xenobiotics.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Over the 5 year life of this Project Licence, it is estimated that 4,800 mice, 13,750 rats, 450 hamsters and 3,250 rabbits will be used.</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 colspan="3">The majority of animals on shorter term studies are expected to have mild adverse effects such as slight weight loss or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more significant adverse effects. Humane endpoints will be adopted or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively</td></tr>
</table>

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

	less adverse effects. The majority of animals will be humanely killed at the end of a study; investigations may include sampling of various organs and tissues followed by microscopy to evaluate potential changes, and detailed examination of parents, foetuses and offspring.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present there are no scientific and legally acceptable evaluations of systemic toxicity that will satisfy regulatory requirements other than use of animals, though validated *in vitro* tests for specific organs are used wherever possible. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies. Where available, sensitive analytical techniques (eg Dried Blood Spot analysis) may be used to reduce animal numbers. Wherever practicable, the combination of endpoints eg general toxicity, reproduction and developmental toxicity, safety pharmacology, mutagenicity in studies is considered, to reduce overall animal usage.
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.	Species choice and use of specific animal models is determined by the need to generate regulatorily-acceptable data. Where a choice of species is possible, care is taken to select the most biologically appropriate species, and the species which most closely relates to man. Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare.

Project Title (max. 50 characters)	**Developmental Regulation of Physiological Systems**
Key Words (max. 5 words)	Fetus, Pregnancy, Developmental physiology, Early life programming
Expected duration of the project (yrs)	5 years
Purpose of the project (as in Article 5)[5]	Basic research Yes Translational and applied research No Regulatory use and routine production No Protection of the natural environment in the interests of the health or welfare of humans or animals No Preservation of species No Higher education or training No Forensic enquiries No Maintenance of colonies of genetically altered animals[6] Yes
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the project is to identify the factors regulating physiological development during normal and suboptimal environmental conditions with the ultimate goal of improving pregnancy outcome and offspring health. Previous human epidemiological and experimental animal studies have shown that the pattern of intrauterine growth is an important determinant of adult physiology with impacts on health, disease risk and lifespan. In particular, low birth weight is associated with adult dysfunction and overt disease of the cardiovascular, metabolic and endocrine systems. However, the mechanisms controlling physiological development during early life remain largely unknown.
What are the potential benefits likely to derive from this project (how science could be	The results will advance understanding of the basic biological processes controlling mammalian development with benefits to researchers,

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

advanced or humans or animals could benefit from the project)?	clinicians, other health professionals and the population at large. Overall, the output from the project is likely to improve quality of life, reduce health care costs, increase livestock productivity and generally raise awareness of the early life origins of adult health and disease.
What species and approximate numbers of animals do you expect to use over what period of time?	This application covers the use of five different species (sheep, pigs, horses, rats and mice). For one complete study lasting 6-12 months, 4-7 farm animals are generally used per treatment group, whereas, in rodents, the number is 8-10 to allow for litter variations. In the 5 year course of the licence with multiple studies by several different research groups, use of the following numbers of pregnant animals are anticipated: 750 sheep, 50 pigs, 50 horses 750 rats and 2500 mice together with a proportion of their offspring. Some of the offspring will be studied over periods of 6-36 months depending on species.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Adverse affects are rare, even in animals undergoing surgery under general anaesthesia, and any discomfort is alleviated by use of analgesics and antibiotics. All animals are inspected regularly and most pregnant animals deliver uneventfully but, when problems arise, veterinary assistance is sort. All protocols are either mild or moderate in severity. All rodents are euthanized at the end of the experimental procedures for collection of tissues for experimental purposes. With the farm animals, some are euthanized for tissue collection at the end of the experiments. Following veterinary approval and certification, the rest of the farm animals are discharged from the Act to a farm or market or, in the case of the horses, to new homes.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Developmental programming of physiological systems is multi-factorial involving interactions *in vivo* between the fetus, placenta and mother and between the mother and neonate. Its study, therefore, requires the use of living animals rather than isolated tissues and organs although *in vitro* analyses (eg tissue culture) subsequent to *in vivo* experimentation can increase the data obtained and allow a comprehensive integrated approach from the molecular to the whole organism level. Studies on acutely anaesthetised animals do not provide the time scale for the developmental changes to occur. There is, therefore, no other way for us to obtain the data that we are seeking than by using conscious animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical calculations using existing or preliminary data are used to determine the minimum numbers required for statistically valid results. In designing experiments, controls and experimental groups are

	run contemporaneously and compared with typically four animals in each group. If statistical significance is unlikely, no further animals are used irrespective of the statistical calculations. To maximise data gained from every animal, we archive a wide range of tissues at the end of the experiments to provide material for development of new methods of analysis and/or for complete collaborative studies, which reduces the need for new cohorts of animals. Approximately 10-15% of our publications arise from the use of archival material.
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.	This application covers the use of five different species (sheep, pigs, horses, rats and mice), each of which provides a unique element in addressing the occurrence and mechanisms of developmental programming. Farm animals, unlike rodents, are large enough to be studied before and after birth across multiple physiological systems whereas rodents with their short lifespan are good for identifying critical developmental windows and intergenerational consequences of environmental change. Mice can also be manipulated genetically. These species also differs in litter size, placental morphology, nutrition, metabolic constraints and mechanisms of pregnancy maintenance. By using five different species, common or unifying mechanisms of developmental physiology can be established with applicability to humans, which could not be obtained by use of a single species or by epidemiological analyses of human populations alone. All animals are monitored on a regular basis to ensure their welfare and protocols usually begin with the simplest, least intrusive procedures and move onto to more complex protocols when positive effects are observed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Safety Testing of Medicinal Products Using Non-Human Primates</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Regulatory Safety Assessment Non-Human Primates</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></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>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</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>In order to allow sound regulatory decisions regarding safe human exposure levels to medicinal products, it is essential to conduct a risk assessment by establishing the intrinsic safety profile of a material, and then relating this to the desired exposure of the material in man.
This licence covers the use of laboratory non-human primates for development, manufacture or testing the quality, effectiveness and safety of medicinal products, foodstuffs and feedstuffs or any other substances or products for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening conditions in man.</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>In order to ensure safe use of a material in humans, it is essential to understand its safety profile. The principal benefit of this project is the provision of safety data to facilitate sound regulatory decisions.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>A proportion of animals will be reused after undergoing mild procedures. Approximately 550 animals will be used per year during over the life of this Project.</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 majority of animals on shorter term studies are expected to have mild adverse effects such as slight weight loss or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more significant adverse effects. Humane endpoints will be adopted or dose levels</td></tr>
</table>

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

	reduced if animals show excessive effects. Longer term studies are expected to have progressively less adverse effects. The majority of animals will be humanely killed at the end of a study; investigations may include sampling of various organs and tissues followed by microscopy to evaluate potential changes.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present there are no scientific and legally acceptable evaluations of systemic toxicity that will satisfy regulatory requirements other than use of animals, though validated *in vitro* tests for specific organs are used wherever possible. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies. Wherever practicable and appropriate, the combination of endpoints eg general toxicity and safety pharmacology, is considered, to reduce overall animal usage.
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 non-human primate is used in regulatory toxicity testing only after careful determination that it is the most biologically appropriate species, and that there is no other acceptable candidate species of lower neurophysiological sensitivity. The use of specific animal models is determined by the need to generate data acceptable to regulatory authorities. Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies will be designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare.

Avian, environmental, risk assessment.

- Summarise your project (1-2 sentences)

This project is designed to assess effects of potential environmental pollutants, such as agrochemicals, on representative avian species, in order to support and inform environmental risk assessment and thereby minimise risk.

- 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.

Governments require and the public expects that substances which may enter the natural environment are safe or their hazards well understood. This necessarily involves the conduct of whole-animal toxicity tests such as those included in this project, which form part of a framework of studies designed to investigate potential effects on all components of the ecosystem. Birds represent a major group of terrestrial vertebrates which may be at risk from exposure to environmental pollutants, and so that risk must be adequately characterised and evaluated in order to protect wild bird populations from unacceptable adverse effects.

- Outline the general project plan.

Studies conducted in this project form part of a framework of studies designed to investigate potential effects on all components of the ecosystem; birds represent a major group of terrestrial vertebrates which may be at risk from exposure to environmental pollutants.

In order to refine studies/data requirements in any given development programme, a tiered approach to testing is adopted. Making appropriate use of preliminary dose-ranging data, acute single-dose toxicity is assessed initially. Subacute (dietary) toxicity, ingestion hazard/palatability/ avoidance behaviour and (for microbial agents) toxicity/pathogenicity are also assessed where necessary according to the nature of the test substance and the environmental hazard. Absorption/distribution/excretion studies may be conducted to inform the risk assessment. Finally, chronic tests are conducted to evaluate potential reproductive effects, which can have profound consequences for wild bird populations.

Occasionally, it may be necessary to perform procedures which are regulated under the Act on wild birds temporarily captured in the course of field studies (specifically, the taking of blood samples in order to investigate biomarkers to inform the assessment of any environmental effects).

- 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.

Since ingestion is the most significant route of exposure to environmental pollutants in wild birds, most birds in this project are dosed with the substance under evaluation by direct oral administration or by dietary incorporation. Inhalation exposure can also be important (e.g. spray drift) and in some studies birds may be exposed in whole body inhalation chambers or, in the case of microbial agents, treated by intratracheal

administration.

Most birds used in this project will experience no, or only mild, adverse effects. However, some birds may experience moderate effects, and in the case of acute/subacute studies, severe signs of toxicity and/or mortality are to be expected in a significant proportion of birds. Humane end-points are applied to prevent unnecessary suffering.

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

The principal benefit of the project is the provision of safety data to facilitate risk assessment and sound regulatory decisions on the development/approval of new substances to which wild bird populations will potentially be exposed. By this process, the safety profiles of products under development can be optimised, and unsafe products identified and eliminated, thus contributing to the protection of the environment.

- 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 test species to be used are generally determined by international regulatory requirements and are selected as being representative of wild populations; quail are most often used to assess potential toxicity to galliforms, and mallard ducks to represent waterfowl. For certain study types and/or to evaluate specific types of test substance/formulation it is necessary to use marker species for other groups, e.g. zebra finch (for passerines) or pigeons (for columbiforms).

All available information is reviewed to ensure each specific test is justified and is optimally designed using appropriate treatment levels. Numbers of birds used in each study are generally linked directly to those in published regulatory guidelines; where non-standard test protocols are required, statistical design principles and relevant experience from previous studies will be used to limit group sizes to the minimum commensurate with meeting study objectives.

- 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.

In order to evaluate adequately the effects on wild bird populations of exposure to a given substance in the environment, it is necessary to conduct whole-animal safety/toxicity tests; currently, no scientifically, ethically or legally acceptable non-animal alternatives that would achieve this objective are available.

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

To prevent unnecessary pain/suffering and to refine studies/data requirements in any given development programme, a tiered approach to testing is adopted; this enables the results of initial testing (usually single dose/acute exposure tests) to be used to refine and minimise the subsequent tests in the programme, either through provision of a basis for appropriate dose selection, or in some cases to determine whether a subsequent study is

undertaken at all (either through regulatory/risk assessment triggers or because it may demonstrate unacceptable toxicity and halt the testing programme).

Birds in all studies are observed regularly to monitor condition, behaviour and clinical health. Bodyweight and food consumption, both useful indicators of wellbeing and health, are also monitored. Appropriate humane end-points are applied to minimise adverse effects while achieving study objectives; severe effects are not allowed to persist, and the affected birds are humanely killed where necessary to prevent further suffering.

Identifying interventional targets – kidney disease
Kidney fibrosis, nephropathy

- Summarise your project (1-2 sentences)

This project will facilitate the discovery of mechanisms in kidney fibrosis that could become new targets for the development of medicines to stop the development of end stage kidney failure. Individual components of identified mechanisms will have new potential medicines designed to modulate their action which after testing in human cell cultures will be trialled in animals that have a kidney disease similar to certain types of kidney disease in humans.

- 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.

Almost all types of chronic kidney disease ultimately progress to end stage kidney failure through a conserved process of kidney fibrosis. Once the kidney has less than 10% of normal function the patient either needs dialysis or transplantation to survive. Average graft survival is only 5.8 years and the majority of patients on dialysis do not survive 5 years of therapy. Considerable co-morbidity is associated with either option. Currently while the primary kidney disease can be treated, this does not always stop the development of fibrosis and loss of function. Apart from the use of anti-hypertensive pharmaceuticals to control glomerular pressure which slows progression there is no anti-fibrotic therapy available. Thus over 100 people per million of population require renal replacement therapy each year costing the NHS over £2 billion a year. Subsequently there is an immediate need for anti-fibrotic medicines, which may also be applicable to fibrotic diseases in lung, liver and heart which together account for 45% of all human death.

- Outline the general project plan.

The project plan will have 2 phases.

The initial discovery phase will be to model the major types of kidney disease in man including diabetic nephropathy (30%), glomerular nephritis (20%), hypertensive nephropathy (20%) and tubulointerstitial nephritis (15%) and use a combination of techniques to identify potential new targets in the fibrotic program. This will range from establishing if targets identified from cell culture screens / intellectual reasoning are actually elevated in kidney fibrosis (i.e. confirmation of a target) to target discovery using comparative proteomics (eg. ITRAQ) or micro-array between disease and normal, or between strains of rodents that develop fibrosis in response to an insult versus those which don't.

The second or interventional phase will develop antibodies or small molecules as a result of the data generated in phase 1 which have previously been validated in cell culture systems used to show either protection (i.e. early treatment from disease inception) or remission (i.e. treatment from a point consistent with human clinical presentation) from the same primary kidney disease in which it was identified and specifically the effect on fibrosis progression. On occasion the use of genetically modified animals may be used to establish the validity of a target in the whole kidney.

- 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.

All experimental procedures, whether modelling diabetic nephropathy, hypertensive nephropathy, tubulointerstitial disease or a type of glomerulonephritis will result in the development of chronic kidney disease and kidney fibrosis which will ultimately lead to end stage kidney failure in the animal. This will be associated with uraemia, proteinuria, lethargy and a general state of poor health, the same symptoms seen in man. Some models to be used such as subtotal nephrectomy and unilateral ureteric obstruction require minor surgery and thus post-surgical pain and discomfort may be seen if appropriate analgesia is not applied.

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

This project is expected to identify novel processes involved in kidney fibrosis, which will lead to new medicines to treat patients and improve their quality of life.

- 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.

Mice, rats and rabbits will be used in this project, as these are the lowest animals on the evolutionary scale in which disease similar to human disease has been generated.

We intend to use up to ten different models of CKD to model the main causes of kidney disease in the human population. Whether we choose rats or mice for this phase will depend on which species best mirrors the human disease and the interventional agents available. For the discovery phase we would expect to use groups of around six to eight animals depending on Power calculations per group and typically three time points to look at the various phases of disease. Four control animals will be sufficient at each time point. Thus most will utilise 36 mice or rats or approximately 360 overall.

The number of targets identified per disease type will define the total number of animals used in the intervention phase. Ideally we would like to find at least one target per disease type. For each interventional experiment groups of 12 to 14 animals will be required depending on Power calculations for interventional groups, six to eight for untreated disease groups and four controls. Each study will have preventative and remission arms. Thus each experiment will use approximately 48 animals. If we test one potential new medicine in each disease model we will use 480 animals, although we would expect to have multiple targets to assess. It may be necessary in these experiments to utilise rabbits if using an antibody based therapy. Rabbits and humans have a greater degree of conservation between many proteins than rodents and humans, and thus antibodies designed to target the human protein may only bind sufficiently in rabbit to elicit the desired effect.

All animal numbers will be determined per model using power calculations using variability data determined previously in that species by the applicant. When a model has not been used previously data from the literature will be used. This will ensure we utilise the least number of animals to obtain meaningful data thus preventing unwarranted animal suffering.

- 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.

A significant proportion of this drug discovery work is carried out using cell cultures, with thousands of potential drugs being screened to identify the most promising compounds. However, kidney fibrosis results from complex diseases that involve many different cell types with haemodynamic and inflammatory components. Therefore further testing in animals is essential as there is no alternative but to use animals in which disease symptoms have been induced. To ensure the fewest number of animals are used, only the most effective drugs that have been pre-screened for activity *in vitro* will be examined in animals.

The animals will undergo procedures that may involve injections or surgery and they may experience moderate discomfort as they will experience some symptoms of disease, such as weight loss and uraemia. Anaesthesia and analgesia will be used where appropriate and welfare score sheets used to monitor disease and any pain, to ensure any discomfort is not severe. There are also limits to the number and frequency of any injections and blood sampling. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff working closely with a veterinary surgeon.

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

All protocols will be performed using anaesthesia and analgesics given as and when required to minimise any pain and suffering to animals. Daily monitoring of animals using a distress score sheet will occur to pick up any adverse effects, while regular measurements of serum creatinine will allow us to determine early when an animal is approaching end stage kidney failure and thus it can be humanely culled before the symptoms of kidney failure cause distress. A series of species specific pain monitoring score sheets have been developed to allow recognition of pain and thus ensure optimised use of analgesics.