Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 5

- **Origins and mechanisms of lung disease**

  Lung Disease, Gene, Environment, Treatments

- **Functional roles of the Endothelial Glycocalyx**

  blood vessel; diabetes; kidney disease

- **The survival and connectivity of developing neurons**

- **The role of inflammation in neurodegeneration**

- **The role of inflammation in immune-mediated….**

- **Pathophysiology of Vascular Responses to Injury**

  Atherosclerosis; restenosis; vascular grafting

- **Targeted prevention and therapy of cancer**

  Cancer prevention, cancer therapy, oncogenic signalling pathway, cancer stem cells

- **Understanding neural function after nerve injury**

  Regeneration, spinal cord injury, nerve injury, nerve repair, cell transplants

- **Efficacy and safety of products for the prevention and control of coccidiosis in chickens and turkeys**

  efficacy, safety, parasite, coccidia, poultry

- **Safety Testing Using Dogs and Minipigs**

  Regulatory Safety Assessment Dogs Minipigs

Origins and mechanisms of lung disease
Lung Disease, Gene, Environment, Treatments

Summarise your project (1-2 sentences)

Gene and environment (allergens, cigarette smoke, pollution, diet,) interactions before and after birth play an important role in development of lung disease (LD) (e.g. asthma, smoker lungs).
This project will study how these interactions cause airway remodelling and inflammation and help to find new targets for treatment in lung disease (LD).

- 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.

Although there are good treatments available for dealing with acute infectious or inflammatory LDs, there is still an unmet need to study the origins of airway remodelling/inflammation and develop new therapies that prevent or reverse these processes in LD.

Recently several new genes have been discovered that are associated with LDs, such like asthma or smoking induced chronic obstructive lung disease (COPD). Little is known about their function in the development of normal and diseased lungs from as early in the womb to adulthood.
One of these candidate genes, ADAM33, is associated with airway twitchiness in asthma and loss of lung function in COPD suggesting that it plays an important role the airway remodelling. The environment, (allergens, pollution, cigarette smoke, infectious agents) is another important factor in the development of LD and very little is known how genes and environmental factors interact during LD development.
For obvious ethical reasons it is not possible to study early lung development under different environmental challenges in humans. The animal model is necessary to study the function of LD candidate genes under different environmental challenges and new therapeutic interventions. Findings from the animal studies will be translated back into human cell and tissue culture models.

- Outline the general project plan.

The overall aim is to determine how genetic and/or environmental factors interact to cause the development and progression of LD.

1. We will study candidate genes in genetically altered animals (GAAs) (e.g. mice/rats). These animals either lack the gene or express too much of the gene and its protein in the lungs which can cause changes in the architecture (more vessels/smooth muscles) that lead to abnormal lung function in diseases like asthma.
2. We will expose animals to environmental challenges (e.g. allergens, infections, cigarette smoke, …) that might interact with genes and study the direct effect on LD development.
3. We will administer novel agents that can intervene with these genes/proteins as potential new treatments for LD.
4. We will expose pregnant animals to different environmental agents and study the indirect/direct effect on the developing lungs in their offspring in order to understand mechanisms of passing disease from one generation to the next.

- 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.

Experimental Breeding of genetically altered animals - Animals under this protocol are not expected to exhibit any harmful phenotype. However, it is not possible to fully predict the nature or severity of any potential defect and for all types of animals there will be careful monitoring for possible side effects.

Administration of substances and tissue sampling- Small samples of tissue will be taken for genotyping. Other than in terminally anaesthetised animals dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm.

Treatment with transgene inducing agents **-** Most of these animals fall under the mild severity limit however some mice (e.g. IL-13 overexpressing) breathing symptoms that might fall under the moderate severity limit and will be closely observed for breathing difficulties. Treatment will be limited in length below the danger period of severe breathing problems.

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

Chronic LDs affect about 300 million people worldwide; COPD accounts for approximately 3 million adult deaths per year, which is equal to 5% of all deaths globally in 2005. Therefore, studies to investigate underlying causes and mechanisms of these diseases are of international importance to health.

As part of this project, we will study LD candidate genes and interaction with the environment at different stages of life from fertilisation of the egg to old age and over different generations. This will help us to discover their function and role in health and disease, which will lead to the discovery of novel treatment targets.

- 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 estimate to use about 2600 animals of each species (mouse/rat). These animals are evolutionary very close to human with a similar respiratory system. These animals are commonly used to make changes in genes (genetically altered animals) that are relevant to lung disease. The size of these animals makes them ideal for lung structure and function studies.
We will design our experiments based on pilot studies and will do special statistical calculations to determine the lowest number of animal needed for our experiments to get meaningful and significant results (guidelines by NC3R).

- 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 have used animals for the following reasons:
1. Studies of normal organ development in the presence of different maternal environmental factors cannot be done in human from as early as in pregnancy in the womb to until after birth for obvious ethical reasons.
2. New genes have been recently discovered that are associated with lung diseases.

These genes can best be studied in genetically altered animals in order to discover their function in lung development and disease development as potential new therapeutic targets.
3. Novel therapies for such new targets need to be studied first in animal models before they can be safely used in human subjects.

Parallel to the animal studies we will study disease related mechanisms in human cell and organ culture models derived from human tissue samples and novel findings from the animal models will also be studied in these human experimental models.

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

Protocols for rodent husbandry, and for detection of signs of distress or ill health are well established.
The research procedures and protocols in the project will not exceed the mild to moderate severity level.
Animals will be assessed regularly for signs of distress or ill health.
Any animals showing signs of distress and/ or pain will be killed by a schedule one method.

Handling will be minimised to routine husbandry and the procedures required for the project.

Functional roles of the Endothelial Glycocalyx

- Summarise your project (1-2 sentences)

  An increasing number of brain disorders are caused by an (auto)immune reaction to our central nervous system. In addition, the clinical symptoms of these diseases are often exaggerated by systemic inflammation. The prime goal of this project is to determine how systemic inflammation affects neurological damage and whether manipulation of the immune system has therapeutic potential.

- 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.

  A good example of an immune-mediated neurological disease is Multiple sclerosis (MS), a condition where antibodies and T cells attack brain cells, causing damage to neurons that lead to devastating clinical symptoms such as paralysis. Recent evidence suggests that systemic inflammation results in re-activation of immune cells, which can flare up disease and further impact on the brain. These effects are mediated by factors released from immune cells or immune cells themselves that enter the CNS, where they can contribute to neuronal dysfunction. The biological mechanisms that contribute to this so-called immune-to-brain communication are incompletely understood, and we aim to address the pathways by studying the effect of innate and adaptive immune activation on neurological function using behavioural and biochemical analysis of CNS tissue. Further, recent studies have shown that immunomodulation has therapeutic value for patients with MS, but not all patients benefit from these treatments due to incomplete understanding of the pathogenesis of the disease. In this project we aim to investigate the cellular and molecular pathways that contribute to acute and chronic immune mediated neurological damage and test novel interventions to prevent or halt neuronal damage. In addition, it has become clear that many other diseases of the brain and eye are mediated or accelerated by our own immune system. These include systemic lupus erythematosis, paraneoplastic disorders and macular degeneration. Similar to MS, these neurological disorders are worsened by systemic inflammation. Further understanding of the biological pathways may result in novel or optimized therapeutic interventions

- Outline the general project plan.

We will test if systemic inflammation alters the normal functioning of the brain by utilizing biochemical, molecular, physiological and metabolic assays. These include changes in behaviour, neuronal damage and immune activation of microglia/astrocytes and cerebral endothelial cells. We will use various agents which activate the innate and/or adaptive immune system. These agents include non-neurotropic bacterial or viral pathogens to better mimic infections as seen in humans and specific inhibitors of the immune system to identify biological pathway that explain altered neurological function in response to low grade systemic inflammation. We will also investigate if and how systemic inflammation impacts on acute or chronic immune mediated brain lesions, for example caused by haemorrhage or autoimmune disease. Neuroinflammation due to haemorrhage will be induced via intracerebral injection of blood derived products, including haemoglobin, thrombin and IgG. Autoimmune disease of the CNS will be induced by active immunization against brain-reactive antigens or by passive immunization of brain-reactive antibodies or lymphocytes. We will use species specific behavioural changes to monitor subtle changes in the brain, including test to assess depression and anxiety and functional imaging to detect neuronal changes in the retina. Agents that activate or suppress the immune system before or after neurological disease, or transgenic animals

will be utilized to gain insight into the biological mechanisms.

- 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.

Animals will be exposed to agents, administered either systemically or directly into the CNS that will induce metabolic, physiological and behavioural changes. These may include paralysis, reduced vision, reduced activity and reduced motor strength. Animals will be carefully monitored for adverse effects.

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

Understanding of the biological mechanisms underlying immune-mediated neurological disease may result in novel or optimized therapeutic strategies. These diseases, which include multiple sclerosis and lupus are devastating disorders, and disease-modifying drugs are desperately needed. Modulation of systemic inflammation may prevent or reduce neuronal damage, but also attenuate neuropsychological symptoms such as depression and anxiety. This will improve the quality of life of patients with a chronic inflammatory disease.

- 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.

To provide a better understanding of the processes of how systemic inflammation affect neurological disease this project will over a five year period use about 5, 000 mice and 500 rats.
We will use a multidisciplinary approach, have optimized our analysis methods and will perform power analysis based on previous work from our research group or from the literature.

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

The interaction between the brain and the immune system is complex and cannot be studied without the use of animals. Behavioural changes which inform about subtle changes in the brain or eye are very informative and add value to the biochemical and molecular analysis of cells and tissues. In vitro experiments using neuron-glia co-cultures or retinal epithelial cells will be used to assess the direct effects of inflammatory mediators on certain neural changes, but the complex interaction between blood vessels, blood brain barrier, immune cells/ mediators and the effect on behaviour cannot be studied in cell cultures. Behavioural changes will inform about subtle changes in the brain, before causing the animals to suffer from disease. We will take multiple blood samples during the experiment, to measure the level of inflammation in the circulation, which will reduce the number of animals and give an accurate picture of the kinetics of an immune response

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

  Administration of substances will be performed using small volumes and where appropriate under general anaesthesia and analgesia. Systemic and local inflammation may result in moderate neurological deficits, such as weakness and loos

sensation of limbs, but any animal which shows persistent, marked neurological problems will be killed humanely and immediately

The survival and connectivity of developing neurons

- Summarise your project (1-2 sentences)

We aim to extend our understanding of the molecular and cellular mechanisms that underpin key aspects of the development of the vertebrate nervous system, focusing on the regulation of neuronal survival and the growth and elaboration of neural processes in the mouse model organism.

- 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.

The purpose of this project is to further our understanding of how the size of neuronal populations in the developing vertebrate nervous system is controlled and what regulates the growth and morphology of neural processes by focusing on the functions of factors and receptors that control the survival and death of neurons and factors that control the growth and morphology of neural processes and to ascertain the intracellular signalling mechanisms involved in each case.
The main objectives are as follows:
1. To understand how the survival and death of neurons is regulated during development.
2. To understand how the growth and morphology of neural processes is regulated during development.

- Outline the general project plan.

The project comprises an integrated body of work on the mechanisms that control the survival and connectivity of neurons that focuses mostly on the roles of the tumour necrosis factor superfamilies of ligands and receptors and neurotrophic factors. The work involves primarily studying isolated nerve cells in cell culture. These highly specialized cells will be obtained from humanly killed mice and mouse embryos at different stages of development from either wild type mice or mice with genetic alterations in genes encoding the factors under investigation, their receptors or proteins involved in intracellular signalling and other proteins whose function is potentially relevant to the work of this programme. We will treat the cultured neurons with the factors under investigation and study the survival of these neurons and the growth of their processes under different culture conditions. We will study the molecular mechanisms that mediate the effects of these factors on cultured neurons. We will also study the phenotypes of relevant transgenic mice using histological methods and by quantify the levels of expression of particular genes in dissected tissues. Mice will first be humanly killed to obtain such tissues.

- 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.

Almost all of work of this programme uses cultured primary neurons to address the fundamental issues being investigated. We will breed transgenic and wild type mice to obtain these neurons for culture. Ear notching and/or tail tipping will be required to obtain tissue for genotyping and/or identification. Ear notching should involve only slight and transient pain. Pain from tail tipping will be controlled by anaesthesia. The mice will be closely monitored, and animals exhibiting any unexpected harmful phenotypes will be killed.

Very occasionally, we may wish to administer a small quantity of a substance to living mice that enables us to study an aspect of nerve cell development (for example, to identify which cells are replicating their DNA before dividing or to regulate gene expression in particular transgenic mice). Such substances cause the mice minimal

distress.

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

Improving our understanding of the mechanisms that control the survival of nerve cells is relevant for the development of new treatments for neurodegenerative diseases such Alzheimer's disease and Parkinson's disease. A more complete understanding of the mechanisms that regulate the growth and shape of nerve cell processes may help in the development of new therapies to encourage the regeneration of nerve cell processes following injury or disease.

- 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 great majority of mice included under this licence are included under protocol 3 to permit us to kill neonatal mice by decapitation because this quicker and less stressful than a schedule 1 method. A maximum of 15,000 adult mice and 38,000 neonatal mice for all procedures. The relatively large numbers of animals estimated reflect the size of the research team this project covers (up to 15 individuals) and the fact that some studies are carried out on populations of neurons that are contain very few neurons, especially at early stages of development, hence the need to harvest the required numbers of neurons from more animals in such cases. We are constantly refining assay and analytical methods to improve their sensitivity so as to reduce the numbers of neurons required in various studies. Furthermore, wherever possible, several members of my research team harvest different populations of neurons and other tissues from the same mice so we reduce the number of mice needed to be killed to harvest neurons and tissue. It is routine practice in my research team to plan and coordinate experiments as a group to maximise reduction.

- 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.

It is essential to study primary neurons at different stages of development to address the fundamental issues relating to growth factor biology and signalling in the developing nervous system under investigation in this programme of research. Cell lines are not available for the various kinds of neurons at different stages of development we need to study. Moreover, cell lines are inadequate for ascertaining the physiological and developmental relevance of particular genes and the proteins they encode in the whole animal. Studies of transgenic mice with appropriate targeted mutations in the genes of interest provide the most relevant and convincing evidence for gene function.

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

To identify for breeding purposes which mice possess a particular mutation in their genetic code, it will be necessary to take a very small sample of tissue from live mice. Ear notching should involve only slight and transient pain, and pain from tail tipping will be controlled by anaesthesia.

The role of inflammation in neurodegeneration

- Summarise your project (1-2 sentences)

This project aims to investigate the cellular and molecular mechanism underlying neurodegeneration and in particular the role of (systemic) inflammation on disease onset and progression.

- 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.

The incidence of neurodegenerative diseases is increasing in our ageing population and novel treatments are desperately needed: however the current state of understanding of the molecular and cellular processes underlying neurodegeneration, and the additional effect of systemic inflammation is not yet sufficient to allow clinical trials. Studies in various animal models of neurodegenerative disease will shed light on the pathogenesis that may provide novel or optimized therapeutic strategies.

- Outline the general project plan.

We will use various models of neurodegeneration to investigate the role of inflammation on neural function and apply a set of behavioural test and imaging techniques to make functional measurement of CNS function. Tissue will be analysed for (immune)-cell activation, proliferation and migration. Collectively, we hope that these techniques will provide insight into the cellular and molecular pathways driving neurodegeneration and provide novel targets for therapeutic intervention.

- 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.

Animals will be exposed to agents, administered either systemically or directly into the CNS that will induce metabolic, physiological and behavioural changes. These may include reduced activity, reduced vision, and/or reduced motor strength. Surgical procedures may induce infections that delay recovery of wound healing. Animals will be carefully monitored for adverse effects and sterile equipment used to prevent them.

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

Understanding of the biological mechanisms underlying neurodegeneration and the effect of systemic inflammation on this process may result in novel or optimized therapeutic strategies. Alzheimer's disease, Parkinson's disease and age-related macular degeneration are devastating diseases for both patients and their carers and due to increasing incidence in our ageing population, disease modifying drugs are desperately needed. Modulation of systemic inflammation may result in reduced neuronal damage, as well as fewer neuropsychological symptoms such as depression and anxiety. This will improve the quality of life of patients with a chronic disease

- 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.

To provide a better understanding of the processes of how systemic inflammation affect neurological disease this project will over a five year period use about 5, 000 mice and 500 rats.
We will use a multidisciplinary approach, have optimized our analysis methods and will perform power analysis based on previous work from our research group or from the literature. We have used a broad spectrum of techniques to maximise the information that can be obtained from our animal models including histopathology, immunocytochemistry, confocal and electron microscopic imaging, quantitative imaging software, qPCR, proteomics, genomics and other molecular and cell biology techniques. By taking advantage of new methodologies we are able to use small tissue samples and thus minimise the amounts of tissue needed.

- 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 not aware of any alternative methods that do not use animals which allow study of the interaction of two complex systems: the central nervous system and the immune system and/or examine the degeneration of selected circuits in the CNS at early stages of disease. The complexity of the pathogenesis of neurodegeneration and the poorly predictive nature of therapeutic interventions are too great for mathematical modelling to replace the use of experimental animals.

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

Using a combination of in vivo cell tracing, selective lesions in relevant structures, telemetry and behavioural assessment we can establish the functional consequences of loss of neuronal connectivity prior to overt clinical symptoms. Administration of substances will be given in small volumes and where appropriate under general anaesthesia and analgesia. Systemic and local inflammation may result in moderate neurological deficits, such as weakness and loos sensation of limbs, but any animal which shows persistent, marked neurological problems will be killed humanely and immediately

- **Summarise your project (1-2 sentences)**

This project will identify which parts of the gel lining blood vessels are most important in health, whether the same parts are damaged in disease, and whether regenerating this gel can improve the function of blood vessels and organs in these disease states.

- **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.**

The inner surface of every blood vessel in the body is covered by a specialised gel, which allows blood vessels to retain important substances like fluid and protein inside the blood stream. This gel becomes damaged in a number of the world's major diseases, such as diabetes, kidney disease, and blocked arteries (atherosclerosis). This stops blood vessels from working normally in these diseases, which in turns stops whole organs from working properly – leading to the complications of these diseases (kidney failure, blindness, heart attacks and strokes). We have found that this gel can be reconstituted by a number of drugs and other substances, but it is not known whether regenerating this gel repesents a new method for treating these diseases. This work will address that uncertainty.

- **Outline the general project plan**.

We will examine the function of whole organs and single blood vessels in animals with and without these key diseases. We will test whether restoring the gel lining these blood vessels improves the function of the blood vessels and organs in these animals.

- **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 function of animal's organs (e.g. kidney, blood pressure) will be measured, and then diseases that model the human condition will be induced using well-established and refined techniques. Whole organ function will be re-measured, and then animals will be anaesthetised before the gel lining individual blood vessels is examined. The expected adverse effects relate to the disease models – for example, increased urine output and weight loss in diabetes.

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

By testing which parts of the gel are important and which parts are damaged in disease, we hope to identify new targets for drugs that could restore blood vessel function. By

testing whether current gel-restoring agents improve blood vessel function in disease, we aim to demonstrate that it is possible to protect blood vessels in these diseases with gel-restoring therapies. We anticipate that this will offer new opportunities for developing new drugs that will be useful in many human diseases.

- **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 intend to study about 10-12 animals per week in this project. In total, we anticipate using ~750 rats, ~2400 mice, and ~1000 zebrafish.

- **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 cells isolated from blood vessels to test whether regenerating agents are active, but the gel is very fragile and is only present and functional inside blood vessels in the living animal. It is therefore necessary to study animals.

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

Wherever possible, we minimise distress to the animals. We achieve this by using minimally harmful ways to study the animals whilst they are conscious, using the most refined methods available. Where necessary to study the function of the gel in models of human disease, we use animal models in rats and mice that are again highly refined, and wherever possible these diseases cause subtle changes that do not have any impact on the animals overall health or wellbeing. We study blood vessels and the gel lining when the animals are deeply unconscious (anaesthetised), and we ensure that the animals do not recover once these procedures have been performed. The only exception to this is in studies on zebrafish larvae, which are studies that we are developing to reduce the need to use rodents like rats and mice. We examine as many aspects of the gel as possible whilst the animal is anaesthetised, using the best available methods that provide very detailed information about the gel, and we are therefore able to substantially reduce the number of animals in the study.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Pathophysiology of Vascular Responses to Injury</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Atherosclerosis; restenosis; vascular grafting</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project aims to increase our understanding of the biological mechanisms that underlie the development of the most common vascular diseases. If we can understand these processes better then it may lead to new and better therapies that can benefit patients by prolonging and improving their quality of life.
Heart attacks are the largest single cause of death in adults, worldwide. The cause of death is the lack of blood flow to the heart via the coronary arteries. Underlying the attack is a disease of the arteries, atherosclerosis, in which deposits (called plaques) of fatty substances, cells and tough fibrous proteins accumulate in their lining. Due to the arrangement of the arteries, if one becomes blocked, a significant part of the muscle making up the wall of the heart becomes deprived of blood, and therefore also deprived of oxygen, leading to the clinical condition of a heart attack. Similar blockages in other arteries can result in stroke or peripheral artery disease. However, these blockages can be bypassed by inserting a piece of vein or artery (performed at the time of surgery), or squashed out of the way by inflating a balloon to expand a fine mesh tube inside the blocked vessel, a technique called stenting. However, often it is found that the treated blood vessel starts to occlude again, and so the effects of treatment may be limited. How these processes are controlled, and how they may be prevented or reversed, is the goal of our research.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td>In our studies involving animal tissues they are removed from animals immediately after death and</td></tr>
</table>

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

project (how science could be advanced or humans or animals could benefit from the project)?	taken to the laboratory, allowing us to study the biochemical pathways involved in the development of atherosclerosis and vascular disease. However, if we are to develop better therapies, then we need to study tissues from animals in which vascular disease occurs in response to clinically relevant stimuli and where the full range of biochemical processes contributing to the disease process takes place. In these cases, we will explore vascular disease, either following injury to arteries or following bypass grafting; this will be done as a surgical operation, under anaesthesia, and animals will receive pain killers before recovery and thereafter. Following this we may administer medicines to the animals to try to alter the development of the disease or to cause established disease to regress; wherever possible these will be given in the food or water, but in some cases will need to be given by injection. For long term administration of medicines they are given by a minipump system that is implanted under anaesthetic and allows their prolonged delivery. We will monitor animals closely during the study. At the end of the study, animals will be killed and tissues removed for further study and analysis.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, rats and rabbits can be used to obtain large numbers of cells that can be grown and studied in the laboratory, enabling us to investigate which approaches are most likely to be effective in a relatively small number of animals. We expect to use up to 500 mice, 50 rats and 100 rabbits in this way during the five years of the project. The mouse is a very important model for studies of diseases of the heart and blood vessels, and an important factor is the ability to study the process in animals in which there has been an alteration of one or more specific genes. In addition there are a large number of reagents and chemicals available that allow very intricate studies of the processes that occur in the cells of mice, so permitting us to study the processes within the cells. In some studies we need to make use of rats because their arteries are large enough to permit us to squash the plaques by means of very small angioplasty instruments. We expect to use 5000 mice during the five years of the project, mainly for breeding of genetically altered animals. Our studies into the pathogenesis of disease in bypass grafts are mainly performed in pigs. These grafts have very similar dimensions, are subjected to similar haemodynamic stresses and develop disease over a similar time course to those in humans. They are the best available model of graft disease and treatments effective in this model

	are likely to be effective in humans. More importantly, they offer an important translational step in the evaluation of new treatments as they may also identify treatments that are unlikely to be effective in humans prior to clinical testing. We expect to use 250 pigs during the course of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	No serious adverse effects are anticipated, though we will take care to guard against wound infection after surgery. Animals will be killed at the end of experiments by an approved method, and in most cases we will harvest tissues and cells from them for further laboratory study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Laboratory studies using isolated cells and tissues will form a major part of the proposed studies and will be used to determine potential interventions in advance of any work in live animals. However, the complex biochemical changes that occur in vascular disease cannot all be modelled in isolated cells or tissues, because they are influenced by a wide range of physiological factors that are unique to living animals. The study of whole tissues or cells isolated from those tissues enables us to refine our studies, because certain interventions and agents can be used that would not be possible (for reasons of toxicity, rarity or cost) in living animals. It also enables us to reduce our use of animals because many cells can be isolated from a single tissue and used for multiple studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	**The number of animals will be minimised by conducting initial studies in cells and tissues in the laboratory, with strictly controlled conditions so as to minimise experimental variability. Our extensive experience of such studies means that we can use historical data to perform power calculations to ensure that the experimental designs are biologically and statistically rigorous. In general the experimental design will involve comparison of a control group with one or more intervention groups using statistical tests appropriate to the data. We will regularly review our designs in the light of the data generated to ensure that the results are statistically rigorous but do not involve the use of unnecessarily large groups 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	Genetically altered mice that develop severe vascular disease are virtually unique amongst experimental animals in that they develop spontaneous unstable atherosclerosis, the root cause of most cases of myocardial infarction in

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	humans, so we have chosen them for our studies of the biological basis of this disease. As far as we can tell, the processes of vascular smooth muscle cell accumulation in mice are similar to those in humans. Comparison of biochemical pathways in mouse and human vascular cells reveals few differences, so mice can be used for such studies. Experimental injury to blood vessels is difficult to perform in mice and often results in a blood clot rather than lesion formation. For this reason, some arterial injury studies will be performed in rats. Like mice, rat smooth muscle cells appear to be very similar to those in humans so these studies are expected to provide valid and useful information that can be translated to the clinic. We have developed significant expertise in the study of inflammatory cells harvested from different pathological sites in mice and rabbits, either from sites of vascular lesion formation, or as part of a foreign body reaction to implanted sterile sponges. These methods generate large numbers of cells for laboratory study, thus reducing the number of animals required, but some results will need to be validated in whole animal studies. **Where possible, pigs will be housed in social groups (either in neighbouring pens, or the same pen) prior to surgery, and upon full recovery from surgery. However, there may be times when the pig cannot be housed socially (e.g. during follow-up assessments).**

Targeted prevention and therapy of cancer

- Summarise your project (1-2 sentences)

We will use genetically altered mice as well as mice with transplanted tumours to investigate roles of cancer gene pathways and test anti-tumour agents by modulating these pathways.

- 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.

Prevention and therapy for most of human cancer are still a huge challenge although substantial progress has been made in the past. Recent advances in understanding molecular mechanisms of tumour development have made it possible to target specific molecules for cancer prevention and therapy. Mutations identified in the PI3-Akt-mTOR signalling pathway are one of the major genetic defects in human cancer. Current available agents for treating tumours associated with aberrant activation of this pathway are very limited in terms of efficiency. It is necessary to identify more effective targeting agents and strategies for tumour therapy.

- Outline the general project plan.

We plan to identify and characterise roles in tumour development of the PI3K-Akt-mTOR and related cancer gene pathways by breeding animals with specific genetic defects and also by tumour transplantation to mice. We will use these mouse models to test candidate anti-tumour agents and to understand the mechanisms underlying response of tumours to agents.

- 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 procedures include breeding and maintenance of genetically modified mice, transplantation of tumours to mice and testing of anti-tumour agents. The major adverse effect is tumour burden caused by tumours either developed spontaneously or transplanted. Another potential adverse effect may be toxicity caused by anti-tumour agents.

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

Anti-tumour agents and strategies identified in this project will help design clinical trials. Information obtained from this work will also advance our understanding of mechanisms underlying drug activity and resistance in tumour therapy.

- 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.

About 4000 mice are estimated to be used within next 5 years. The mouse is one of the most suitable small mammals for human tumour research. Both genetically altered mouse models and models with transplanted tumours have been very useful in tumour drug discovery. The genetically altered models are particularly useful because they

develop tumours in an intact immune system and use the natural tissue environment. The models with transplanted tumours are also useful because they provide an easy and quick approach to test drug effect and also capacity of tumour cells to initiate tumour formation and promote tumour progression. As discussed below, to minimise the number of animals to be used, *in vitro* tissue culture studies will be used extensively and studies will be properly designed and analysed with assistance of statistical methods.

- 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 will use alternative *in vitro* systems to achieve our research objectives wherever possible. Animals are needed to confirm the pharmacological activity of anti-tumour agents *in vivo* for various reasons. First of all, the pharmacological effect of an anti-tumour agent on cultured cells may be different from that on animal tumours *in vivo,* although *in vitro* testing is useful to rapidly identify candidate therapeutic agents. In addition, the effect of agents on cell proliferation and growth rate can be assessed *in vitro* but animal models are needed to test whether the agents can cause tumour shrinkage or eradicate the tumours. Furthermore, it is necessary to assess potential adverse effect of therapeutic agents in animals.

To reduce the animal numbers, extensive testing of therapeutic agents in cultured cells will be performed. Pilot studies involving a very small number of animals will be performed in the case of new agents to be tested. Studies will be properly designed and analysed with the assistance of a trained medical statistician to ensure that the maximum data output can be obtained by using the fewest animals. The use of live imaging will also help reduce animal numbers.

To minimise pain, suffering, distress or lasting harm, the least invasive procedures will be used for the minimum amount of time. The animals' condition will be frequently monitored including weight loss and tumour burden by trained staff. If any signs indicate that pain, stress, suffering or lasting harm is caused or significant weight loss or tumours reaches an unacceptable size, the animals will be humanely killed. Substances administered should have little or no detrimental effect on the health of the animals. In some cases, effective doses have been described in details in the literature. On occasions when new agents are to be tested, stepwise tests will be used, starting with a low dose and using no more than two animals per step.

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

  To minimise pain or distress caused by tumour growth, animals will be carefully monitored daily by qualified and experienced technicians. Tumour burden will be limited to the minimum required for a valid scientific outcome. Humane end points are well defined according to tumour burden as well as the general condition of animals together with specific clinical signs caused by anti-tumour treatment. Animals will be humanely killed immediately if any signs of suffering appear.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Understanding neural function after nerve injury</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Regeneration, spinal cord injury, nerve injury, nerve repair, cell transplants</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Traumatic nerve injuries such as spinal cord injury can produce devastating and long-lasting effects in patients, with symptoms ranging from loss of sensation, paralysis, chronic pain, incontinence and loss of sexual function. The significant disabilities that result from such injuries are not only detrimental to the quality of patients' lives, but also place a large social, emotional and financial burden on carers and the health system. Currently there are limited effective treatments for spinal cord injury. Despite recent advances in this area of research, few therapies have been translated to the clinic. To date we still do not completely understand the pathological processes that occur after nerve injury and in particular, what molecular mechanisms can promote growth pathways in the injured nerves and encourage them to regrow. Therefore, more research is required to understand and identify the pathways that increase regeneration of injured neurons. The knowledge gained from these studies may give rise to therapeutic opportunities for development into innovative medicines that can be used to treat nerve injuries.</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>As we do not completely understand the growth processes that contribute to nerve repair, this work is expected to contribute to the body of knowledge concerning the molecular mechanisms of regeneration and help us gain insight into the fundamental biology of nerve function. This may lead to the identification of novel experimental therapies for treatment of spinal cord injury. The study of cell transplant strategies will provide</td></tr>
</table>

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

	valuable information which may help the scientific community weigh the benefits and the risks of these cell transplants in spinal cord injury and other neurodegenerative diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Rodents are relatively low order sentient animals and are the most commonly used species for studies in nerve regeneration. Over the course of the 5-year programme, we anticipate that 175 mice and 250 rats will be used in procedures described in the project. Genetically modified mice will also be used to help us evaluate the physiological importance of specific gene function.
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?	Evaluating experimental medicines for diseases requires that the studies be carried out in animals with the relevant disease or injury. Injuries to nerves in the peripheral nervous system (eg. sciatic nerve) or central nervous system (eg. spinal cord) will be generated in a controlled manner in mice and rats. These injuries are discrete, affect only a restricted part of the central nervous system and do not impinge on special senses. As repair does not normally occur, this will inevitably result in some loss of function for the animal. However, without such animal models it is not possible to fully understand the pathophysiology of spinal cord damage. We will exercise care and caution during our procedures and ensure that the injuries do not exceed defined degrees (moderate severity). Treatments in the form of small molecules, replication-deficient viral vectors and cell transplantation may be delivered to the injured rodents by direct injection into nerves, brain or spinal cord and following treatment return to function will be assessed using behavioural tests. At the end of the experiment, animals will be killed humanely and the tissue analysed to evaluate the effectiveness of the treatments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The pathology of nerve injuries is complex and involves intricate interactions between different cell types in a complex biological environment of the nervous system that cannot be replicated in a tissue culture dish. We will carry out extensive basic and mechanistic investigations using *in vitro* and cellular models prior to, and in parallel with, animal experimentation. However, there are no non-sentient systems that model the mammalian somatosensory system and can replace the use of animals. Animal models provide a relevant system in which genetic manipulations can be performed and regenerative responses can be measured by using anatomical, behavioural and electrophysiological techniques.
2. Reduction	The structure and function of the peripheral and

Explain how you will assure the use of minimum numbers of animals	central nervous systems of rodents are well-characterised and functional outcome measures such as behavioural testing and anatomy that have been developed in these species are accepted by the scientific community as relevant and appropriate methods of assessing nerve regeneration. Pilot *in vivo* experiments comprising small group numbers will be undertaken before definitive experiments. We will also seek to reduce the number of animals studied by careful experimental design, the adoption of sensitive outcome measures with small variation and the study of only the most relevant time points.
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 nerve injury models described are well studied, characterised and are recognised within the scientific community as relevant models for studying neural regeneration. While there may be some loss of function after injury, we will ensure that motor impairment and individual protocols will not exceed defined degrees to prevent any distress to the animals. We will also use analgesic drugs after surgery and apply the minimum number of somatosensory tests to yield unambiguous results. The behavioural tests that will be carried out in conscious animals are essential for assessing functional recovery. Such tests do not produce damage to tissue and therefore there will be minimal pain, distress and lasting harm to the animals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Efficacy and safety of products for the prevention and control of coccidiosis in chickens and turkeys</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>efficacy, safety, parasite, coccidia, poultry</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></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 overall aim of the programme of work is to provide efficacy and safety data for products for the control and prevention of coccidiosis in chickens and turkeys. Parasite control products are continually being developed, but it is a legal requirement for these to be fully tested for safety and efficacy prior to them being marketed. This licence will enable studies to be carried out on behalf of pharmaceutical companies to satisfy these legal requirements.</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 overall aim of the programme of work is to develop safe and effective means of controlling coccidiosis in chickens and turkeys. Disease and ill health caused by coccidiosis in poultry continues to be a worldwide welfare concern. This problem is being exacerbated by the rising levels of resistance to various products.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Chickens and turkeys. Over the five year life of the project we might use a total of 15000 birds.</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>In the majority of cases the adverse effects are likely to be minimal or mild. Where animals are challenged with coccidiosis in order to test the efficacy of a product, then the disease model will be the least severe available in order to satisfy European guidelines. In addition, the animals will be monitored frequently and we will intervene when the severity limit is being approached. Where at all possible, animals will be returned to farm. Where</td></tr>
</table>

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

	this cannot occur (an unregistered veterinary product for example), animals will be humanely euthanased.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	When testing efficacy and safety of veterinary medicines the European guidance documents require that the target species of animal is used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where there is a European guidance document detailing the requirements, we will comply with these. Where there is no guidance document, we will take the advice of a statistician.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The animal species we propose to use are as dictated by European guidance documents. In many cases the adverse effects are likely to be minimal or mild. Where adverse effects are anticipated, animals will be monitored regularly to ensure that severity limits are not exceeded. Where severity limits might be exceeded, we will intervene to treat the animal.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Safety Testing Using Dogs and Minipigs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Regulatory Safety Assessment Dogs Minipigs</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>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>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 dogs and minipigs to evaluate the hazard profile of xenobiotics in terms of general 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>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>Over the 5 year life of this Project Licence, it is estimated that 5,200 dogs and 1,050 minipigs 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>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 less adverse effects.</td></tr>
</table>

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

	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. 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, safety pharmacology, mutagenicity etc 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.