Home Office

# Animals (Scientific Procedures) Act 1986

## Non-technical summaries granted during 2013

## Volume 31

## Project Titles and key words

- Infectivity of TSE Agents

  Variant Creutzfeldt-Jakob Disease. Iatrogenic transmission, Assessment of clinical risk

- Small animal models of infection

  Infectious disease, therapy, vaccines

- Studying the role of macrophage in tumours

  Cancer, zebrafish, angiogenesis, macrophages

- Mechanisms of progressive IgA nephropathy

  IgA nephropathy, kidney disease, progression.

- Mechanisms of particles toxicity

  Mesothelioma, nanoparticles, toxicity, asbestos, carcinogenesis

- Safety of Biopharmaceutical Medicinal Products

  Biosafety, extraneous agents, QC testing

- Disorders of haem metabolism and links to disease

  Haem, inherited diseases, drugs

- Novel Immunotherapeutic interventions for cancer

  Immunotherapy, solid cancer, localising agents,

- Imaging of Cardiovascular Diseases

  CMR, cardiac perfusion, ASL, CAD

- The interactions of innate and adaptive immunity

  Immunity, adaptive, colitis, infection, metabolism

- Tissue Remodelling in the Uterus and Placenta

  Endometrium, Placenta, Oxidative stress

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Infectivity of TSE Agents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Variant Creutzfeldt-Jakob Disease. Iatrogenic transmission, Assessment of clinical risk</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>1 year</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[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>The objective of the project is to assess the risks of transmission associated with surgery or transplantation that involves patients infected or potentially infected with vCJD or other transmissible spongiform encephalopathy agents (TSEs)</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>It is important to quantify the risks of TSE infection that are associated with surgery and transplantation of tissues. Once the potential infectivity of a range of tissues is known, then official guidelines can be issued to the medical profession that will minimise the risk of transmitting such potentially lethal infections to naïve patients</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>No more than 10 mice will be used in the lifetime 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 animals will have been injected with tissues from patients known to have vCJD. This procedure was conducted under a previous licence and the mice will now continue to be maintained with daily observation so that any clinical signs of infection can be detected. At the first signs of disease the animals will be humanely killed.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement<br>State why you need to use animals and why you cannot use non-animal alternatives</td><td>Despite the continuing development of in vitro assays, the bioassay is still the most sensitive method of demonstrating the presence of low levels of infectivity. Previous experience from work conducted at this establishment has shown that some samples that appear negative by these assays will still produce signs of disease when</td></tr>
</table>

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

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| | injected into mice. Material from infected patients that were used to challenge animals were available in only small amounts and potentially have such low levels of infectivity that standard detection systems such as Western blot would not provide a signal from the sample. For risk assessment strategies the challenge model using animals is the only way to characterise the spread of the agent from the point of original infection. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | As this licence is requested solely to maintain existing experimental groups, no further animals will be added under this licence. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | The strain of mice used in this work has been selected to provide the optimum sensitivity and rapidity of progression to disease for vCJD and to provide well characterised brain lesions that are proven to confirm TSE infection. The mouse strain has been shown in previous literature and in our studies to be most sensitive to CJD infection and will allow demonstration of the presence of infectivity at very low levels that might otherwise escape detection. In addition, the sensitivity of this model increases the confidence that negative results are a true demonstration of an absence of prions. Use of these mice will allow us to compare the results of our work with our previous data and with that of other workers in the field. All mice will have been housed in a social group throughout.<br><br>The project team has a wealth of experience in assessment of clinical signs of TSE infection in mice and there are well characterised end-point criteria enabling humane intervention at early time points that have been shown to be predictive of disease whilst minimising any suffering. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Small animal models of infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Infectious disease, therapy, vaccines</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
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<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim of work to be conducted under this licence is to provide a capability to develop small animal models of infectious diseases where these are not associated with a large on-going programme of work.<br>In each case the objectives will include some or all of the following:<br>1. To study the pathogenesis of an infectious disease<br>2. To validate the model as appropriate for use as a model of human infectious disease<br>3. To investigate, where necessary, the immune responses to infection and relate them to those seen in man<br>4. To develop models for efficacy testing of novel vaccines or therapies<br><br>For example, the licence currently includes a protocol to investigate the occurrence of antibiotic resistant clinical isolates of Chlamydia trachomatis, and another to aid in the design of an effective Chlamydia vaccine. This is an increasingly common sexually transmitted disease in humans and can lead to infertility in a number of cases. Justification and protocols for developing other infection models will be added by amendment as they are required and will be listed separately under the appropriate sections of the licence.</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</td><td>All work conducted under this licence will be to investigate areas where there is an unmet clinical need. For example The work proposed for Chlamydia infection here addresses two scientific issues.</td></tr>
</table>

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

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| project)? | • Antibiotic resistance; the occurrence of antibiotic resistant *Chlamydia* infections is highly contentious, and if demonstrated here in a well controlled and well defined model system, could have a significant impact on the treatment of human *Chlamydia* infections.<br>• Vaccine development; the development of an efficacious vaccine is considered essential for the control and future elimination of *Chlamydia* infections in humans. The work described here is an important component of an ongoing programme to identify vaccine candidate proteins. |
| What species and approximate numbers of animals do you expect to use over what period of time? | It is anticipated that the majority of work undertaken will use mice or, occasionally, rabbits. In previous cases under an earlier licence, the majority of models were established using one of the extensive range of inbred mouse strains, hamsters or rats as appropriate. The number of animals used will vary according to the type of evaluation required but in every case statistical advice will be taken on the minimum number of animals that will give significant, meaningful results. |
| 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? | Under the previous licence in all cases it was possible to establish clinical scoring systems that allowed early intervention to avoid undue suffering and progression to severe disease, and given the extensive experience gained during that time it is expected that the same criteria will apply to work conducted under this licence. Animals given an infectious agent are likely to show clinical signs of disease and the severity of these signs will vary with the agent but at most will be moderate. Some animals may show weight loss, ruffled fur, depressed appearance and, occasionally, withdrawal from the rest of the group. A combination of these signs will be used to determine a humane end point that is predictive of progression to disease |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | In the majority of cases, substantial preliminary work will have been undertaken in laboratory-based assays that do not involve the use of animals. The animal work that is required will generally be the last stage in the process, where confirmation of appropriate immune responses are required or proof is needed of the protective efficacy of a vaccine or therapy. In these cases it is impossible to reproduce the complex immune responses that occur either during infection or as |

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| | a result of vaccination by using cell culture or computer modelling. In many cases the use of animals can give valuable information on vaccination and boosting strategies, the best route of application and the best adjuvant that can be safely used to boost the vaccine effect. In some cases the appropriateness of a particular animal model of disease will be well documented in the scientific literature, such that there is no need to use further animals for objectives 1, 2 and possible 3. However, if the disease is novel or not well characterised, then these two objectives will require preliminary work in animals. Objectives 1, 2 or 3 will not, however, be utilised out of context of a new therapy or vaccine that needs to be tested (i.e. a new model of infection will not be set up without a potential therapy being available to test). |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | The Establishment has access to bio-statistical advice and extensive experience in establishing new animal models. In every case during the planning stage statistical advice will be taken on the minimum number of animals that will give significant, meaningful results. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | The species of animal to be used will depend upon the disease to be studied and current information available in the literature. It is anticipated that the majority of work undertaken will use mice, rats, hamsters or, occasionally, rabbits.<br><br>In all cases robust schedules of clinical monitoring will be established for each disease model based on extensive experience of animal models over a range of species such that humane end-points are clearly established to minimise suffering. This experience will also serve to identify critical periods when more frequent monitoring is required. All animals will be housed in social groups with enrichment provided as appropriate for the species. |

| Studying the role of macrophage in tumours |
| --- |
| Cancer, zebrafish, angiogenesis, macrophages |

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

We will induce cancer formation in zebrafish to understand how cells called macrophages help cancers develop.

- **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 macrophage is a cell that patrols the body and eats dead cells and bacteria. However, human cancers contain many macrophages and it seems that macrophages actually help cancers grow, perhaps by helping to develop a blood supply. We do not understand how this happens, and understanding this is the aim of this licence.

- **Outline the general project plan.**

Our work generally follows three stages. Firstly we cause tumour development in zebrafish, secondly we alter gene function or use drugs treatments, and thirdly we observe the tumours to measure whether this has an effect on cancer 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.**

To perform this work, we need to cause cancer in our zebrafish. We will do this by injecting cancer cells or genetic alteration to make tumours form. We will then use genetic manipulation or drug treatments and observe the zebrafish under microscopes to see what effect these have. The fish will experience some discomfort during tumour development, although this is generally limited. Drug treatment or genetic manipulation has the potential to cause some unpredictable adverse effects; such animals will be closely monitored and such studies will not continue if harm is produced. Some of our drug treatments need to be administered in the dark, which may induce some distress, although it appears to be well tolerated. Other procedures will be carried out under anaesthesia or are painless.

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

Our work will greatly improve our understanding of how macrophages help cancers to grow and so may lead to new treatments for cancer patients

- **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 use up to 17800 zebrafish; most of these will be adults used simply to breed and so will not undergo painful experiments. Many of the zebrafish we use will be less than five days old and capable of only limited distress due to limited development of the nervous system. We will use the least number of animals by ensuring we use the most efficient experimental designs and by observing the same animals on repeated occasions

rather than groups of animals examined on single occasions
.

- **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 need to study the interactions of many cell types within an intact organism (tumour cells, macrophages, blood vessels etc). There is currently no way to replace animals for such studies; we will continue to look for such ways to replace animal studies completely.

Our use of zebrafish, and particularly zebrafish at very early stages of development, represents refinement as without these models our experiments would need to be performed in higher organisms such as mice.

Since we can observe the same animals on repeated occasions (without inducing distress) this will greatly reduce the number of animals needed.

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

We have chosen the lowest possible organism to perform such work, in order to cause the least possible suffering. These animals will be closely monitored and all painful procedures will be carried out under anaesthesia. Animals will be monitored closely in conjunction with dedicated animal welfare staff and a veterinary surgeon.

| Mechanisms of progressive IgA nephropathy |
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| IgA nephropathy, kidney disease, progression. |

- Summarise your project (1-2 sentences)

  IgA nephropathy is a common form of kidney disease worldwide, with around 30% of patients progressing to kidney failure requiring treatments such as dialysis. We aim to better understand how this disease progresses in order to identify targets that can ultimately be targeted to slow or stop this process.

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

  IgA nephropathy is characterised by deposits of a protein, Immunoglobulin A (IgA), on the sieving apparatus of the kidney, the glomerulus. Around 30% of patients have progressive disease leading to kidney failure necessitating dialysis or transplantation, which themselves are associated with far greater rates of death compared to the general population. There are currently no specific treatments to slow or stop this progression.

  A characteristic feature of IgA nephropathy, in common with many other forms of kidney disease, is the abnormal appearance of protein in the urine due to damage to the sieving apparatus. It has been established that the presence of protein in the urine is not only a marker of kidney disease but is also a cause of progressive kidney scarring, which eventually leads to kidney failure.

  The precise type of protein responsible for damaging the kidney is unclear. Our laboratory experiments show for the first time that the IgA molecule itself is able to exert powerful effects on tubular cells which line the early part of the drainage system of the kidney, causing the release of chemical messengers known to drive the process of inflammation and scarring. We propose that IgA, that escapes from the blood into the urine through the damaged sieving apparatus in IgA nephropathy, subsequently causes these harmful effects.

  In this project, we plan to examine in detail the interaction between IgA and tubular cells, to determine how this might cause kidney scarring.

- Outline the general project plan.

**Phase 1:**
To identify key pathways activated by IgA in proximal tubular cells
To determine the specific structural properties of the IgA molecule that lead to its inflammatory and scarring properties.

**Phase 2:**
To establish a mouse model of progressive IgA nephropathy
To examine this model using mice genetically deficient in candidate IgA receptors or components of their signalling pathways to see if they are protected against progressive disease.

**Phase 3:**
To translate findings based on Phase 2, for their applicability to human disease.

- 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.

IgAN will be induced in mice using an established published protocol. Mice will be fed a protein in the drinking water to induce IgA production, and will subsequently be injected with the same protein, resulting in formation of IgA containing immune complexes, which then deposit on to the kidney causing damage. Selected mice will also undergo an operation to remove one kidney before the injections, to accelerate this process.

**Expected adverse effect**

- Pain killers will be given to the animal to ensure that any pain following the operation is minimised.
- According to previous studies, the kidney disease induced by this protocol should not be severe enough to cause symptoms. However if animals do display any symptoms related to kidney failure, i.e. anorexia, severe weight loss or dehydration, it will be killed to prevent suffering.

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

By examining the interaction between IgA and the proximal tubule, we hope to identify receptors or signalling pathways that can be targeted. The ultimate aim is to develop treatments to slow or even stop the process of kidney scarring in patients with this condition, preventing patients with IgA nephropathy reaching end stage renal failure.

We will also aim to identify factors, for example expression of a certain IgA receptor, that make patients more susceptible to progressive kidney disease in IgA nephropathy. These may be helpful in enabling clinicians to more accurately predict outcome at time of diagnosis, or to tailor specific treatments to those groups.

- 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 expect to use up to 300 mice for this project.

This mouse model of IgA nephropathy is currently the most refined model of progressive IgA nephropathy. It is used by investigators in many centres and results in a predictable disease course with minimal suffering to the mouse. Using this model of IgA nephropathy will allow us to examine genetically altered mice who do not possess candidate receptors for IgA or their downstream signalling pathways, which will give us valuable information about the interaction of IgA with the proximal tubule.

Initially, small numbers (up to 5 per group) of mice will be used in pilot studies, where IgA nephropathy will be induced, with or without nephrectomy. These mice will be accurately characterised, so that dosage of the administered antigen can be optimised before carrying out experiments on the transgenic strains, minimising the total number of animals to be used.

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

In Phase 1 of this project, we will conduct cell culture experiments to identify as much information as possible about candidate proximal tubule IgA receptors and important signalling pathways, to take forward to the mouse model.

Although these cell culture experiments will provide valuable information, they imperfectly mimic the behaviour of the kidneys for the following reasons:

1. Proximal tubular cells are exposed to a number of proteins (known and unknown) simultaneously following damage to the sieving apparatus of the kidney which will be impossible to mimic in cell culture.
2. Only one surface of proximal tubular cells is exposed to filtered IgA, and this cannot be modelled perfectly in cell culture.
3. It is likely that interactions between proximal tubular cells and other cell populations are important in driving kidney scarring and these cannot be reliably modelled in cell culture.

For these reasons, we have elected to study this mouse model in addition to the cell culture experiments.

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

The protocol stated has been published by other groups previously. It is expected that the degree of renal impairment induced will fall well short of that expected to cause symptomatic renal failure. However, mice will be observed closely for signs of symptoms associated with renal failure and will be killed if severe symptoms develop to prevent suffering.

The uninephrectomy will be carried out be a trained skilled operator, and animals will receive analgesia to prevent post-operative pain.

| Mechanisms of particles toxicity |
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| Mesothelioma, nanoparticles, toxicity, asbestos, carcinogenesis |

- Summarise your project (1-2 sentences)

The research aims to uncover how particles interact with the body and how to prevent their harmful effects. A better understanding of particle toxicity may also help to improve existing treatments for patients exposed to asbestos in the past.

- 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.

During life course the human body is exposed to particles used for manufacturing modern materials. Some of these particles (e.g. nanotubes) are similar in their physical and biological characteristics to asbestos, a well known cause of human lung diseases. The most severe illnesses are asbestosis, malignant mesothelioma and lung cancer, and they usually manifest themselves after 20-40 years of latency. There is a raising concern that manufactured nanoparticles may drive toxic effects leading to diseases. This has a potentially enormous public and occupational health impact, given the dramatic increase in the manufacture of nanotubes. The overall aim of the project is to gain understanding of how particles interact with the body and how to prevent their harmful effects.

The objectives are:

- examine the mechanisms of toxic effects of particles on exposed tissues and explore potential drivers of carcinogenesis.

- use the information gained in objective 1 to prevent toxic effects and disease and to improve existing treatments for patients with the diseases caused by exposure to pathogenic fibres.

- Outline the general project plan.

We will examine effects of pathogenic fibre in cellular models using an array of profiling techniques and create a map of critical pathways and target molecules responsible for harmful effects in normal cells. These can give us some knowledge about processes that we are interested in. We will use cell cultures for as much of our analysis as possible and only after that these pathways will be validated *in vivo* experiments. The molecules verified as driving inflammation/carcinogenesis in mice will be then modulated *in vitro* in cellular models by using agents that we think may help to prevent or reduce fibres particle toxicity by means of manipulating gene expression, protein expression and/or activation. Only when we have good results in cultured cells (i.e. reduction or prevention of toxic effects in the cellular system) will we test our findings in mice.

- 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.

In this project animals will be exposed by delivering fibres by intrapleural, itraperitoneal or i/v injection into the cavities covered with mesothelial cells- these are the targets for particle toxicity. Human disease has an unusually long latency after a period of exposure, during which disease develops. To understand this mechanism, we will use low doses of fibres, usually over a period of 5 days to mimic a working week and in some experiments a slow release of these agents from the mini-pumps or via i/v cannulation. Thereby animal will receive lower concentrations of particles/agents, and that will enables us to observe the changes over a prolonged time, mimicking a long latency period in human malignant mesothelioma. This is tolerated by animals quite well and expected adverse effects are very rare and related to a delivery method. The severity does not exceed moderate, however in our experience over 95% of animals experience no more than mild effects.
For therapeutic modulation of toxic effects we will mainly use agents with known properties and dosing regimen, therefore expected adverse effects are rare and severity is mild. In case of a new drug with unknown adverse effects, small pilot studies will be conducted to refine the experimental design and achieve the desirable effect with the lowest possible dose, thereby minimising adverse effects.

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

In general, knowledge gained by this research may help to prevent or reduce adverse effects of particles. A better understanding of particle toxicity will be beneficial for public health in the context of massively growing manufacturing of nanomaterials. It may also improve existing therapies for the diseases caused by fibres exposure. This would have the potential to benefit people who had been exposed in their lifetime.

- 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.

Previous work conducted with collaborators looking at the wider effects of nanoparticles has been successful and we now wish to take the experimental work further by focusing on the molecular mechanisms involved in the areas of risk identified. We are planning to use approximately 6000 animals over 5 years of the project. Mice and rats are proposed for the studies because there is an advantage of using transgenic mouse models; and rat model of inflammation is considered to be the gold standard for in *vivo* work and also has higher than mouse susceptibility to mesothelioma.

- 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 minimum number of animals will be used consistent with predicted statistical significance. Colonies of mice will only be continued until the consequence of experiments has been confirmed and will be kept to the minimum size consistent with good practice on breeding genetically altered mice. In addition, a major part of the project will be done *in vitro*. Mechanistic and morphological testing will be conducted in human cells lines before modulation in animals. This not only refines, reduces and replaces animal work, but ensures that animals are only used in a targeted way to verify the role of molecules shown to be significant *in vitro*. This portion of the work is already being conducted but ultimately cannot substitute completely for *in vivo* experiments.

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

The protocols included in this project will allow us to achieve objectives of the study and are designed to serve this purpose. The suggested models based on using these protocols have high biological relevance and high expected benefits. The severity bands are mild or moderate and any animals showing signs exciding these levels will be humanly killed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Safety of Biopharmaceutical Medicinal Products</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Biosafety, extraneous agents, QC testing</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>1 year</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[5]</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>
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<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 objective of the project is to ensure the safety of biopharmaceutical products through the use of animals to:<ul><li>assay for potential contaminating agents</li><li>produce antisera for virus seed neutralisation and serological assays</li><li>validation of in-process steps for reduction / removal of TSEs derived from material of human or bovine origin</li><li>characterisation cell lines for vaccine production.</li></ul>Biotechnology has long been used to produce medicines for human or animal use in the form of vaccines and monoclonal antibodies and other new technologies such as gene therapy, xenotransplantation and transgenics are in the early stages of development. However, with such products there is a risk of contamination with microbiological organisms which may be endogenous or latent in the animal of origin, or which may be introduced from animal raw material or other sources during the production process.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The benefits of the project are evident in that the testing will help to ensure that biopharmaceutical medicines for human or animal use are free from contamination and other safety risks such as tumorigenic potential.</td></tr>
<tr><td>What species and</td><td>The species and approximate numbers expected to be used over the 5 year licence period are as</td></tr>
</table>

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

| | |
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| approximate numbers of animals do you expect to use over what period of time? | follows:<br>Mouse = 13,240<br>Nude mouse = 780<br>Hamster = 1,610<br>Rat = 450<br>Nude rat = 50<br>Guinea pig = 1,110<br>Rabbit = 45<br>Chick = 120<br>Chick embryo = 9,100 |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | The overall likely / expected severity level of the project is moderate. Adverse effects are most likely to result from toxicity of the test material or the presence of an extraneous agent in the test material. In addition, where challenge and positive controls are used, effects of these are expected to be observed and will be closely monitored against humane end-points detailed in the licence. All animals will be euthanased using a schedule 1 method. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | In conjunction with *in vivo* studies, *in vitro* testing is performed as an important part of the submission package.<br><br>Where possible, *in vitro* testing will be performed, however, current legislation for the safety of biopharmaceuticals also requires *in vivo* testing. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Consultation with regulatory authorities and clients will determine the number of animals to be used on a case by case basis for pre-studies and susceptibility studies. Any background information from *in vitro* and *in vivo* data shall be reviewed. Since studies are compendial the option for reduction is limited.<br><br>Where possible (i.e. negative control groups) animals will be shared as common to several studies. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Each study protocol shall be reviewed on a case by case basis, and particular attention made to inoculation routes, dose volumes and an assessment made of likely adverse effects. The study protocol shall also include daily clinical assessments of all animals. Advice from the named veterinary surgeon and named animal care and welfare officer may be requested prior to and during the study. |

| Disorders of haem metabolism and links to disease |
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| Haem, inherited diseases, drugs |

- Summarise your project (1-2 sentences)

The aim of this project is to try to understand how some drugs or other chemicals cause liver and nervous system diseases in some people with certain inherited defects but not in others with the same genetic differences.

- 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 use of oxygen in the body employs a natural chemical, a constituent of haemoglobin for carrying oxygen in blood, of cytochromes for burning of food for energy and of enzymes that make hormones and detoxify chemicals and drugs. However, in certain genetic diseases the formation of this chemical in the body, especially the liver, can be severely disturbed, often triggered by drugs and foreign chemicals, leading to liver disease followed by toxicity of the nervous system and skin photosensitivity. This does not occur in all people who have the gene changes, many are unaffected, so that other factors including food and other inherited changes must be influential. The precise symptoms depend on a combination of the genetic propensity and exposures to drugs and chemicals which also may be constituents of the diet. The research aims to discover new understandings of how this happens to help susceptible patients and prevent such attacks.

- Outline the general project plan.

It is well established that many of the liver problems caused in these inherited diseases can be investigated with mouse models using drugs or chemicals but the precise molecular changes in genes or pathways leading to liver damage are still not understood. In patients the precise liver damage can lead on to adverse effects in the nervous system and skin. Increasingly, new mice with changes in genes are becoming available together with new concepts of how gene actions can be controlled. We have previously created a new mouse gene model in the pathway which though showing no overt harmful defects in those mice carrying one copy of the changed gene will be invaluable in exploring these mechanisms. Experimental procedures in mice will designed to model the human conditions and seek genetic and molecular changes to be analysed by state of the art techniques to produce testable hypotheses for the sporadic nature of the overt disease symptoms in people.

- 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.

Mice (normal and genetically modified) will be treated with drugs and at levels which are known from previous studies to have mild effects. The mice used with modified genes that show physical changes have already been documented in the public literature. Due consideration will be given to potential outcomes when combining treatment with mouse gene models and the various routes of drug administration. Adverse effects are anticipated to be mainly seen as physiological perturbations and increased levels of natural metabolites without overt physical changes. Tissue samples from liver and other organs will be taken for analysis by advanced molecular technology.

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

These studies will advance our understanding of a critical fundamental physiological pathway and at the same time help to understand why clear disease is seen in some

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| patients with particular inherited conditions and not in the majority. |
| • 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.<br>• The number of mice used will be approximately 2500 over the course of the licence. There is a substantial literature on this species modelling the human conditions of interest and increasing opportunities to use of generated genetic models.<br>• The minimum number of animals will be used based on the known statistical frequency of responses judged from experience and the literature. |
| • Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives.<br>• Where appropriate, say how you will use non-animal studies in parallel with the project. Where possible In vitro studies will be used to complement these mouse models but it is well established that they can give interesting leads but at the present time cannot replicate findings in complex physiological environments which may include response in one organ depending on another. |
| • Explain why the protocols and the way they are carried out should involve the least suffering.<br>• Protocols are designed to be the least severe and with the minimum of physiological changes. consistent with obtaining clear statistically significant results. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Novel Immunotherapeutic interventions for cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunotherapy, solid cancer, localising agents,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td>Yes</td><td></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>Our project aims to use novel minimally toxic localised immunotherapy treatments to inhibit all stages of cancer.<br><br>The overall aim of our project is to test the effectiveness of these novel immunotherapeutics against tumour growth in established murine models of cancer.<br><br>Within the overall aim, we have four objectives for the research<br>1. To test the effectiveness of these novel immunotherapies compared with the same immunotherapeutics in a non-localised form, on tumour progression, and anti-tumour immunity in mice.<br>2. To confirm that these agents are not detected in the blood of the animals (i.e. systemically).<br>3. To identify anti-cancer therapies (including vaccines) that can work in combination with the above agents to boost efficacy.<br>4. To study how solid cancers such as prostate cancer can weaken the immune system -so that we can also discover new targets that can be treated with immunotherapy</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>We believe that this project could identify effective low cost immunotherapies suitable for all stages of cancers that can potentially save millions of lives.</td></tr>
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| advanced or humans or animals could benefit from the project)? | |
| What species and approximate numbers of animals do you expect to use over what period of time? | We propose the use of mice, which have immune systems that are similar to that of humans. We anticipate that 5000 animals will be used for the duration of the licence. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Animals will be injected with tumour cells so that tumours grow either as a mass or within the lungs. Animals will be monitored frequently to ensure that they are not in pain or distress and we will use an established acceptable end point for experiments based on a maximal tumour size, and not death of animals. We anticipate very few adverse effects but, if these are seen, treatment will be ceased and/or animals will be culled humanly |
| **Application of the 3Rs** | |
| 1. Replacement<br>State why you need to use animals and why you cannot use non-animal alternatives | To achieve the objectives of the project, it is vital to use animal models: The tumour environment is complex and agents that activate immune cells to kill tumour cells in-vitro may not work in humans. Although slight differences occur between murine and human immune systems, mice challenged with tumour cells are the most appropriate model for screening of preclinical efficacy of our drugs. Many of our reagents, such as cytokines have an equivalent effect on murine and human immune cells. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | We will reduce animal numbers in 3 ways:<br>**1)** We will only use agents in-vivo that are confirmed as being active in-vitro for boosting tumour specific immunity This will reduce animal numbers at least 5-fold as some of our preparations will be inactive in-vitro<br>2) Our experiments have been designed with the help of a statistician to use the lowest number of animals which will give statistically valid results.<br>3) We will have detailed write ups and record keeping for all experiments noting all observations on animal health and welfare. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | **Choice of Models**:<br>The murine tumour challenge models we are using have been established for many years as the simplest way of assessing primary and metastatic tumour progression, with minimal suffering to the animals and obtaining maximal amounts of data.<br><br>**Minimizing Suffering**<br>We will undertake a number of refinement |

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| | measures for our experiments to minimize animal suffering and therefore minimise variations in our measurements.<br>1) For injections of cells or other therapeutics, we will use needle sizes with the maximal gauge possible for delivery of the agent/s so that the animal suffers the least amount of pain.<br>2) For injections, the area to be injected will be treated with ELMA cream to minimise discomfort during and after injection.<br>3) Body condition will be monitored as a whole using a number of criteria such as weight loss (or weight gain combined with thinning of the animals), condition of fur, lack of activity, signs of respiratory distress and alertness, compared to non-tumour-induced control animals in the same experiment. |

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Imaging of Cardiovascular Diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>CMR, cardiac perfusion, ASL, CAD</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td></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>Diseases of cardiovascular system remain a major health care problem requiring novel diagnostic techniques as well as therapies. Computer simulations and laboratory work initially demonstrated the efficacy of new techniques however animal models have become key tools to replicate these in patients. Preclinical models using animals, which bear anatomical similarities, has allowed development in investigating feasibility and ex-vivo and in-vivo models prior to human studies. Experimentation in the preclinical setting allows use of gold standard techniques not possible in human studies. Microspheres validation translates directly into the clinical application of those models.<br>Non-invasive imaging methods that allow visualization, quantification and monitoring of subclinical disease and therapeutic intervention are of increasing value for the assessment of the systemic and local disease burden before and after treatment. Due to the excellent soft tissue contrast, high spatial and temporal resolution as well as the tomographic nature of magnetic resonance imaging (MRI), anatomy and function can be assessed with unique accuracy and reproducibility. These features minimise the number of animals required to measure statistically significant changes when comparing novel treatments, devices or contrast agents. Explanted hearts used in two- or four-chamber physiological models provide flow and pressure controlled environment for replicating and monitoring conditions resulting in myocardial perfusion deficiency.<br>Physiological modelling or development of new</td></tr>
</table>

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

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| | imaging techniques for myocardial perfusion quantification requires validation methods, of which usage of microspheres is still the gold standard. Even non-invasive techniques such as ASL (arterial spin labelling) have to be validated before their clinical application. Animal studies are capable of this and a pig model bears close anatomical and physiological resemblance to enable subsequent transfer of information gained into human studies. |
| 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)? | This project will provide better understanding of cardiovascular diseases, possibly allowing in the future for early diagnosis, patient management and prognosis of such conditions. Additionally, the development of new cardiac MRI techniques can then be used on a clinical basis to benefit patient care. MRI is now a well-established and widely used technique in patients displaying several advantages including unrivalled spatial resolution and the lack of ionising radiation. CMR perfusion has been shown to be superior to nuclear-based perfusion imaging and is increasingly used as the non-invasive and radiation free test of choice to guide clinical decision-making. The ability to non-invasively assess myocardial blood flow and the degree of coronary artery disease has significantly changed the risk-stratification and management of patients. Novel approaches to assess myocardial perfusion quantitatively have emerged but require validation before being introduced into routine clinical practice.<br>Animal models possess physiological similarities to a human heart and are established as a means of validation in the preclinical setting before human studies are considered. Microsphere injections are considered the gold standard for perfusion measurements however must be performed immediately prior to sacrifice. The use of microspheres is precluded in humans but has been accepted in animal models.<br>On this basis, a number of animal experiments is essential to enabling development of new sequences that we believe would benefit a significant number of patients. |
| What species and approximate numbers of animals do you expect to use over what period of time? | This project uses animal models for validation of quantification processes of imaging techniques. As a primary tool we use MRI (magnetic resonance imaging), which provides high signal-to-noise ratio and high tissue specificity contrast. All data will be collected using high-resolution MRI techniques, which, with combination of the usage of gold standard validation techniques, reduces the number of animals used in this project. In the case of MRI development, the sequences will have been tested |

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| | thoroughly in synthetic objects that possess magnetic properties (known as an MRI phantom). Each animal study will be performed carefully with attention to collect high quality data. This will ensure the minimum number of animals is sacrificed during the project. We estimate that over the project duration 75 pigs will be sacrificed. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Due to the nature of this project - harvesting a vital heart - animal suffering is reduced to minimum and there are no adverse effects expected as the anaesthesia is terminal. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | The animals will only be used as a last step when the simulation of cardiac physiology or pathophysiology is required. In the case of MRI development, the sequences will have been tested thoroughly in MRI phantoms then in the cardiac perfusion phantom for initial validation. The animals will only be used for validation purposes before clinical applications, which is a necessity. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Minimum number of animals will be used, reducing it by using high quality imaging technique providing reliable results. The usage of gold standard validation techniques reduces the number of animals used in this project. The well-controlled two- and four-chamber explanted porcine heart approach allows us to simultaneously collect data from multiple cardiac conditions, further reducing the number of animals used. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Porcine hearts are similar in gross structure and coronary arterial and venous anatomy to hearts in humans. Models of acute and chronic disease can be created in a similar way and time frame to human pathology and therefore the scope for information is thoroughly applicable to human conditions. The anatomical similarities allow MRI scanning to be comparable in terms of methods and time delays (and other parameters) in setting up new sequences. The new technique can then easily be transferred to a human setting. The animals will not be left in pain at any point and will be given general anaesthesia without undue distress. |

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| **Project Title** (max. 50 characters) | The interactions of innate and adaptive immunity |
| Key Words (max. 5 words) | Immunity, adaptive, colitis, infection, metabolism |
| Expected duration of the project (yrs) | 5 |
| Purpose of the project (as in Article 5)[11] | Basic research: Yes ~~No~~<br>Translational and applied research: Yes ~~No~~<br>Regulatory use and routine production: ~~Yes~~ No<br>Protection of the natural environment in the interests of the health or welfare of humans or animals: ~~Yes~~ No<br>Preservation of species: ~~Yes~~ No<br>Higher education or training: ~~Yes~~ No<br>Forensic enquiries: ~~Yes~~ No<br>Maintenance of colonies of genetically altered animals[12]: Yes ~~No~~ |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | We aim to understand the molecular pathways that link different cell types of the immune system that are responsible for causing diseases such as autoimmunity, cancer and diabetes and are involved in protection from infections. |
| 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)? | We anticipate that the findings from this project will help us to discover targets for the treatment of a number of human diseases such as inflammatory bowel disease, diabetes and obesity and certain forms of cancer. This will form the platform for developing new drugs and diagnostic tests for these disease |
| What species and approximate numbers of animals do you expect to use over what period of time? | We will be using mice and intend to use in the region of 4500 in a 5 year period for immune system experiments. We also plan to use 500 mice over 5 years in various dietary and metabolism experiments. This would be an approximate usage of 1000 mice per year for experimental purposes. We also have 3000 mice allocated for breeding over the 5 years. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | A number of our procedures are rated as mild severity and adverse effects from these are considered unlikely (1% or less). The adverse effects from our moderate procedures are all listed and we believe adequately controlled. Possible or expected adverse effects would include poor response to surgery or to injections , although these would normally not occur very frequently. Also possible are poor response after radiation treatment, colitis induction or tumour growth experiments and the acceptable limits for all of these are clearly outlined and experimental animals will be carefully watched and removed from studies |

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

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| | if any concerns develop. Animals will be culled by approved schedule 1 methods at the end of experimental procedure or if it becomes necessary to alleviate suffering at any time. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | There are currently no lab techniques that can accurately simulate the entire innate and adaptive immune system and this means that animals are required for this kind of research. Our group is active in attempting to use lab based tests to simulate various individual aspects of the immune system where possible in order to try and minimise the requirement to use animals. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | We are committed to maximising the amount of experimental information we obtain from each individual experimental animal, enabling us to hopefully minimise usage. We routinely use single strains of mouse in multiple research areas and use multiple organs from the same mouse in order to keep animal usage down. We are also trying to minimise the amount mice we breed for each research area. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | We are using mice as they have an immune system comparable in complexity to humans and are the most frequently used model for the human immune system. The use of genetically modified mice in our proposed program of work allows the maximum amount of experimental data to be obtained in the most efficient manner. We are mindful at all times to minimise welfare costs to the animals we use. All of our protocols are designed to minimise any welfare costs and in the event that it is unavoidable animals are monitored closely |

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Tissue Remodelling in the Uterus and Placenta</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Endometrium, Placenta, Oxidative stress</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">During human reproduction the uterus and the placenta undergo profound growth and remodelling. This is particularly striking in the endometrium which is dynamically regulated and is shed and regrows every month. Growth factor expression profiles relevant to normal endometrial remodelling and endometrial diseases have been previously defined. However, a detailed molecular description of the function and the regulation of these cellular mediators is lacking. We will define the function of the genes that control these processes using mouse models in which the endometrium grows and regresses. We will compare this with endometriosis tissue in mice and women. These processes may be altered in some patients and in some cases this can lead to cancer. It is not known which genes are the most important in this process so we will use genetically modified mice with endometriosis to test several candidate genes. We will characterize the cellular and molecular processes that underlie uterine aging.<br><br>Blood vessel growth and the effects of changes in the blood and oxygen supply are important in placenta. The growing baby depends on the maternal blood supply. We know the placental cells invade and change the maternal blood vessels that supply the placenta but we still do not know how the growth and migration of these placental cells is controlled. Defects in these processes early in pregnancy lead to the ‘Great Obstetrical Syndromes’ - miscarriage, preeclampsia, intra uterine growth restrictions and stillbirth. In the UK</td></tr>
</table>

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

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| | approximately 1 in 200 babies are stillborn.<br><br>Therefore to improve our understanding of the mechanisms that underlie placental cell growth and migration we will use genetically modified mice to test genes that regulate the growth of the placenta and its response to stress (lack of oxygen or changes in nutrition for example).<br><br>We already know that changes in the oxygen level in the placenta leads to changes in the placental proteins released into maternal circulation and also a reduction in fetal growth. Once we identify these proteins (in humans) we will test their function genetically modified mice to determine whether their loss causes a reduction in fetal growth. |
| 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)? | We will characterize the cellular and molecular processes that underlie uterine aging. Since women are exposed to contraceptive steroids this may have important consequences for public health policy.<br>Candidate diagnostic or therapeutic targets for the treatment of endometriosis may be identified.<br>This work will add to our basic understanding of the mechanism underlying the association between the presence of endometriosis and some forms of ovarian cancer. This may lead to the development of new diagnostic or screening tools for use in women.<br>Measurement of the changes in secreted placental proteins may be useful as diagnostic tests and natural antioxidants (which we have previously identified) may by useful in treating premature babies. |
| What species and approximate numbers of animals do you expect to use over what period of time? | We plan to use no more than 10400 mice, most of which will be genetically altered, over the course of the 5 years planned for this work |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Most of the planned studies will use tissue collected from genetically modified mice. It is very unlikely these animals will show signs of suffering. Some mice will undergo surgery, for example ovariectomy or abdominal surgery to inject material into the uterus. This is of moderate severity but good surgical techniques, anaesthetic and pain relief will be used during and after surgery to minimise adverse effects. At the end of the study the mice will be humanely killed. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | While the richness of the complexity of endometrial and placental growth and function cannot be replicated *in vitro,* some aspects can be studied *ex vivo* and *in vitro*. Therefore cell and tissue culture models will be used wherever possible and before |

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| | animal studies commence. Indeed the majority (approximately 80%) of my current work uses *in vitro* methods. The use of protected animals is necessary as the complex interaction between multiple cell types present in the endometrium during decidualisation and regression and in the placenta cannot be replicated *in vitro*. Uterine aging is dependant on the complex interaction of numerous cells and factors over a prolonged period which cannot be modelled *in vitro*. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | All mice will be housed in high health status facilities. This reduces environmental variability and so makes the results more consistent and this means we can use fewer animals. Wherever possible we will make several measurements of the growing lesion in the same animal. This type of data is statistically more powerful so again we can use fewer animals. We have collaborators who are specialist statisticians who will provide advice on the minimum numbers necessary and analysis methods. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Models of decidualisation are known that do not require surgery but only in primates whereas mice are well-studied and allow precise genetic manipulation permitting detailed mechanistic studies to be completed.<br>The use of ovariectomized animals ensures a consistent and reproducible response.<br>Where possible we will use tissue and time specific genetic modification which will minimise the chance of severe phenotypes.<br>In animals that undergo surgery close attention will be paid to surgical technique, anaesthesia and analgesia. |