Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 10

Project Titles and key words

- Inflammatory response to nanoparticles

  Inflammation, Nanoparticles, Exposure

- Regulation of pancreatic islet hormone secretion

  Diabetes mellitus, treatment, insulin, glucagon

- Fertilization and early development in mammals

  Fertilization, egg, IVF, embryo, mouse

- Assessing the risk of pesticide exposure to wildlife

  Pesticides Environment Birds Mammals Exposure

- PATHOPHYSIOLOGY AND THERAPY OF AUTOIMMUNE UVEITIS

  intraocular inflammation, uveitis, immunomodulation, retinal imaging, autoimmunity

- Controlling the adaptive immune response

  Immunology, Dendritic Cells, Lymph, Cell Migration

- Mechanisms of axon degeneration and its delay

  Axon degeneration; Alzheimer's disease; motor neuron disease; peripheral neuropathy; ageing

- Nutrition for sustainable cattle production

  Cattle, nutrition, forage, environment

- Fish Movements and Behaviour

  Fish, Movement, Behaviour, Telemetry, Otolith

- Sero-prevalence of Schmallenberg in sheep in the UK

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Inflammatory response to nanoparticles</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Inflammation, Nanoparticles, Exposure</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></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>Engineered nanoparticles are an emerging category of materials engineered to be smaller than 100 nanometres because of the useful properties conferred by their small size but there are concerns that some of these properties may be hazardous to health. Engineered nanoparticles are increasingly used in many aspects of everyday life, which increases the potential for exposure and health risk. This work will study the inflammatory response in various biological systems following exposure to engineered nanoparticles. Initially the research will be focused on inflammation of the lung, after inhalation of engineered nanoparticles, as this is considered to be the major route of exposure. Inflammation will be studied in broncho-alveolar lavage fluid (BAL), organs and excreta samples to quantify inflammatory cells and mediators, proteins and gene expression. Blood will be isolated to study the systemic inflammatory effects.</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>Increased knowledge of the intracellular mechanisms that contribute to the development and maintenance of inflammatory processes. This knowledge will;
contribute to our understanding of the interaction between engineered nanoparticles and biological systems and any pathological consequences
produce data urgently required for assessing engineered nanoparticles health risks
help in the design of safer engineered nanoparticles.</td></tr>
<tr><td></td><td></td></tr>
</table>

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

What species and approximate numbers of animals do you expect to use over what period of time?	250 mice 250 rats
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?	*Toxic effects are considered unlikely or minimal because the engineered nanoparticles to be administered are already being used in domestic products and are those considered particularly non-toxic at larger particle sizes or because the studies will not be of sufficient duration for any malignant symptom to develop.* *Animals will be monitored during and immediately after administration of the engineered nanoparticles and at least on a daily basis thereafter.* *Occasionally the animals may be restrained and/or housed individually which will cause distress and so a moderate level of severity has been assessed for this work.* *All animals will be killed on completion of the study.*
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	**Whole body systems are essential for the inflammatory processes to reach the full pathological effect required to mimic human exposure to engineered nanoparticles. However, *in vitro* experiments will be used to study the different components and pathways, to help elucidate intracellular mechanisms responsible for the inflammatory response and in the design and planning of the *in vivo* studies.**
2. Reduction Explain how you will assure the use of minimum numbers of animals	**Statistical advice will be sought from specialist statisticians in study design and data interpretation as appropriate, and study design will be informed by accumulating data.**
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.	**Rats and mice have been the animal model of choice at this establishment and elsewhere for similar studies after inhalation of aerosols of larger sized particles (0.5 to 5 µm). Many studies have been performed to characterise the deposition, clearance, temporal organ distribution, excretion parameters and biological effects of radioactive and other potentially hazardous materials in these species.** **Over a number of years' experience of exposing rats to inhaled particles, we have worked to minimise their time in restraint tubes, in order to reduce the stress on the animal. We have also refined our metabolism cages such that when it is necessary to keep these animals singly, we introduce environmental enrichment and make sure that they have visual contact**

	with animals in adjacent cages.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Regulation of pancreatic islet hormone secretion</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Diabetes mellitus, treatment, insulin, glucagon,</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)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td><s>Yes</s></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>Diabetes is a major metabolic disorder. It results from insufficient release of the body’s blood glucose-lowering hormone <u>insulin</u> combined with oversecretion of the body’s principal blood glucose increasing hormone <u>glucagon</u>. Diabetes affects every cell of the body, which explains the wide spectrum of secondary complications. It has been estimated that diabetes costs the NHS £1million per hour.

Diabetes results from a combination of genetic predisposition and ‘environmental’ factors (such as obesity and age). Recent advances in genetics have led to the identification of ~70 gene variants that increase disease risk. The majority of these affect the function of the pancreatic islets, small endocrine micro-organs situated in the pancreas that contain the cells that produce and secrete insulin, glucagon and somatostatin. However, the exact mechanism by which genetic variation in these genes increases diabetes risk and impairs pancreatic islet function remains obscure but such information would probably facilitate translation of these findings into improved clinical care.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>These studies will help to explain the genetic causes of diabetes, highlight signalling pathways that are crucial for normal metabolic regulation of insulin and glucagon secretion and clarify how they become perturbed in diabetes. These pathways may represent suitable targets for novel pharmacological therapy.</td></tr>
<tr><td></td><td>We will use mice for all experiments. The total</td></tr>
</table>

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

What species and approximate numbers of animals do you expect to use over what period of time?	number of mice covered by this license is estimated to be 2000. In most cases, only a small number of mice carrying the genetic defect will be required (20-30 mice/project).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We expect (based on prior analysis of ~30 genetically modified mouse strains) that the functional consequences of genetic modification will be mild (or moderate at worst) and limited to mild hyperglycaemia/diabetes. However, should it become evident that the phenotype is more severe than anticipated, corrective measures will be undertaken or the breeding of that particular mouse strain terminated. This license is for breeding genetically modified mice. All functional tests will be carried out in test tubes with isolated tissues/cells.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many of the experiments will be performed on human pancreatic islets obtained from a clinical islet transplantation programme. However, supply of human islets is limited and some experiments are technically not feasible using human islets. Thus, some of the experiments will be performed on mice that have been genetically modified to mimic the disturbance in diabetic patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will breed mice that express fluorescent proteins in the different cell types within the pancreas. These mice are completely normal except for the expression of fluorescent protein in a small number of cells. This will allow cell identity to be established before the experiments commence. This will increase success rate and thus result in a reduction in number of animals used. Access to such genetically modify mice will thus result in a net reduction of the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the ideal model for this type of work. Mice also have the advantage that procedures for their genetic modification are well established. Tumoural cells are, in general, too dedifferentiated to allow physiological characterisation and elucidation of the expected subtle impact of ablating potential diabetes genes. Use of a small number of experimental animals is therefore needed.

Fertilization and early development in mammals.
Fertilization, egg, IVF, embryo, mouse

Summarise your project (1-2 sentences)

We aim to understand the basic me**c**hanisms that operate at fertilization in mammals and to establish new ways of diagnosing and treating human infertility.

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.

Infertility affects about 1 in 7 couples and the major form of treating human infertility is *in vitro* fertilization (IVF). However, this technique is not always successful for reasons that often remain unknown. One major potential reason why fertilization does not occur is because the egg lacks a stimulus from the sperm. It has been previously shown that this stimulus is provided by a specific protein in the sperm. Our research will investigate how a lack or deficiency in this protein could explain these currently unresolved cases of infertility. We will find ways of making this sperm protein artificially so that it could be used in future to help couples conceive. We will use mouse eggs and mouse embryos as a models for human eggs and embryos.

Another major problem with current IVF treatments is that when fertilization is successful, there may be several embryos generated. It is common practice to re-implant 2 of these embryos into the uterus of the prospective mother. However, this can often lead to twins, and it also increases the chances of triplets. If a mother has twins or triplets there is a greater risk of problems developing during the pregnancy. It would be best to only transfer one embryo but the problem then remains of how to choose which is the 'best' embryo. In our research we will also be studying the biochemical responses in the mouse egg and embryo in the first few days of development. In some cases we make use of specialist imaging methods that do not harm the embryo. At a later stage these could be developed for use in IVF clinics to select the best quality embryos.

Outline the general project plan.

We shall use mouse eggs for our work. These will be obtained from female mice. We will collect and use these eggs, so the experiments as such are carried out on the eggs rather than the mice. The mouse eggs will be microinjected with fluorescent dyes and other molecular probes, as well as sperm proteins. We shall study the responses of eggs using microscopes equipped with specialist cameras.

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.

We will inject mice with hormones so that they make more eggs than normal. The mice are injected twice, two days apart. Then after a further delay of about 15 hours (to allow ovulation) the mice are humanely killed. This procedure involves injecting the same hormones that are used in woman as part of IVF treatment and they rarely, if ever, have any harmful side effects. After the procedure the mice are humanely sacrificed, and eggs collected after dissection.

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

We will advance our knowledge of how a sperm stimulates an egg to develop into an embryo by finding the factors in the egg that allow it to respond to the sperm protein that triggers development. By making a stabilized version of this sperm protein we will provide IVF clinics with a new way of treating couples whose eggs have failed to fertilize. We will also improve our understanding of how we can assess the ability of a human embryo to undergo successful development. This could eventually lead to better methods of selecting which single embryo to re-implant into a prospective mother, and this will in turn help reduce the additional problems in pregnancies associated with twins and triplets.

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 3000 mice over 5 years. We will use female mice for the procedure. Mice are chosen for several reasons. Mice can be made to ovulate many eggs in response to hormone injection. They are the best model for humans for our work since they use the same protein to stimulate development as human eggs and the timing and requirements for mouse embryo development is very similar to human embryos. In fact human IVF clinics now use specialist solutions and plastic-ware, for culturing human embryos, which have been specifically tested for 'embryo-compatibility' using mouse embryos. This can be done because the basic solutions for human embryo culture are very similar to those used for mouse embryos. In response to hormone stimulation mice can produce a relatively large number of eggs compared with other mammals. This reduces the number of animals used for a given study.

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.

There is no 'animal free' cell culture system for making eggs that can develop into embryos. The way in which a mammalian egg develops into an healthy baby is very complex and there are many factors that can affect the formation and health of a baby that go way beyond our basic knowledge. The timing and complexity of the signals in mammalian embryos make them rather different from invertebrate embryos. Hence, the only way to study how factors affect a human embryo is to use another mammalian embryo.

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

The procedure that is carried out under a licence involves injecting female mice in the abdomen with a hormone using a sharp needle, on two separate occasions. The mice rarely, if ever suffer, any side effects or other consequences as a result of this injection other than the production of more eggs than usual.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Assessing the risk of pesticide exposure to wildlife</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pesticides Environment Birds Mammals Exposure</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>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>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td></td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[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 aim is to provide acceptable data for regulatory authorities to allow them to make more accurate assessments of the risks to wildlife posed by pesticides, through studies conducted to:

• Test for the avoidance of pesticide treated food or granules in captive animals

• Field trials of pesticide effects on wildlife

The results will provide information that can be used in deciding on approval for the uses of pesticides, in formulating advice about the choice of environmentally safe compounds, and ensuring that applications are recommended that minimise hazards to wildlife. The results will aid in the selection of those products that cause the least harm, or no effects on wildlife. These studies should lead to improvements in the methodology for such testing. The data generated aims to provide sound information concerning how to provide adequate protection for wildlife, whilst minimising the use and suffering of animals in the laboratory setting.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This work is relevant to the agrochemical and agricultural industries, and to government departments concerned with formulating and implementing policies and regulating the use of pesticides. These benefits cannot be obtained by any means other than by the experimental exposure of animals. Failure to perform these types of tests would result in reduced protection for</td></tr>
</table>

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

	wildlife.
What species and approximate numbers of animals do you expect to use over what period of time?	For laboratory trials no more than 1000 animals per year in total or 500 per year for any one species. Species likely to be used are the wood mouse, bank vole, house sparrow, feral and wood pigeon, common pheasant and grey or red legged partridges. For field studies numbers cannot be predicted as they will depend on numbers caught under field conditions, but from previous experience are unlikely to exceed 1000 animals in total in any one year. Species that may be encountered in the arable/vegetable farm landscape could form part of a monitoring programme.
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?	Testing hazards of pesticides, captive animals- moderate severity. The study design and monitoring time-points should limit the level of exposure and thus reduce the risk. Testing hazards of pesticides in the field – Severe. Birds and mammals may come into contact with and be exposed to potentially harmful pesticides. At the end of the study the animals will either be put back in to stock for breeding or re-use, released into the wild or killed using a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Tests are requested by regulatory authorities when there is believed to be a risk to wildlife and it is not possible to predict the effects of exposure with existing data. The use of live animals is necessary to perform more refined risk assessments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	**Each study is subject to ethical and statistical review. A strong statistical thread therefore ensures that we maximise the amount of robust data collected whilst minimising the number of animals required in achieving the objectives of the study.**
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 used will be those typical of the species found in the arable/vegetable farm landscape and are very good representatives of other species. The data gained for one particular species can necessarily cover another found in the same habitat, unless there are behavioural differences to suggest otherwise. From previous experience it is very rare that there has been a loss due to a poisoning event, to

	minimise pain and suffering the animals are monitored regularly during the course of a study.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>PATHOPHYSIOLOGY AND THERAPY OF AUTOIMMUNE UVEITIS</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>intraocular inflammation, uveitis, immunomodulation, retinal imaging, autoimmunity</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><u>Yes</u></td><td></td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td><u>No</u></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><u>No</u></td></tr>
<tr><td>Preservation of species</td><td></td><td><u>No</u></td></tr>
<tr><td>Higher education or training</td><td></td><td><u>No</u></td></tr>
<tr><td>Forensic enquiries</td><td></td><td><u>No</u></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td></td><td><u>No</u></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>Uveitis is a major sight-threatening disease, the fourth commonest cause of world-wide blindness. This project uses mouse models of uveitis to provide essential information on how uveitis is caused which will allow development of new and better treatments.

Uveitis as a term describes inflammation inside the eye (intraocular inflammation). This can be caused by infections (50% cases) but the remaining 50% cases are caused by disease of the immune system (autoimmune disease) in which the patient’s tissue proteins become the focus of attack by his immune cells (lymphocytes). How this happens is not known. This project uses models of autoimmune uveitis in mice to discover the mechanisms by which the immune cells attack the eye tissues, especially the retina. The work is needed because it offers the chance to develop new therapies such as cell based therapies which will provide hope for patients in saving their sight without the life-threatening side effects of current drugs - immunosuppressants. The aim is to have customised patient specific cell therapy in time.</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 potential predicted benefit from this work is extensive, if a new patient-customised cell therapy can be developed for use in the clinics.</td></tr>
</table>

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

What species and approximate numbers of animals do you expect to use over what period of time?	We will be using mice for our studies as they develop clinically identical disease to humans. We are planning to use genetically modified/transgeneic mice, which do not always develop the required genotype, hence the estimation of 5000 animals during the duration of the project to ensure sufficient mice of the correct genotype for the study. Experiments are planned to obtain statistically robust data, which would take into consideration also biologic variance. This includes meticulous planning of mouse breeding programme and regular frequent checks of the breeding colonies, in order to meet the experimental requirement with minimal mouse numbers. Once particular experiments have been completed certain genetically modified lines may no longer be required and will be cryo-preserved.
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?	There are few significant adverse events expected apart from skin injection site reactions and anaesthetic reactions. Mice have normally very low visual acuity (nocturnal animals, mainly sensing by smell and whisker touch) and are not adversely affected by the eye inflammation. In addition, the eye inflammation is intraocular and painless (as in humans) so there is no external evidence of disease. Surgical procedures such as splenectomy and lymph node removal are conducted under anaesthesia and do not produce debility.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Experimental autoimmune uveitis (EAU) is an animal model of human disease for which there is no in vitro model. Understanding the mechanisms of inflammation and translating them into therapeutic opportunities for medical practice has already reaped benefit for patients and our further work with these models should in long term provide safer treatments which also prevent visual loss in affected patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible (approximately 10% of work) the in vitro techniques will be used which will reduce number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general	The protocols involve procedures performed mainly under general anaesthesia and involve minimal or no suffering to the animal (skin ulceration may occur as a result of immunisation protocol but mice do not experience any distress or pain related to eye inflammation and uveitis). The

measures you will take to minimise welfare costs (harms) to the animals.	immunomodulatory treatments will be administered without anaesthesia; however these do not cause suffering, only temporary discomfort.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Controlling the adaptive immune response</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunology, Dendritic Cells, Lymph, Cell Migration</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><u>Yes</u></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td><u>Yes</u></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The project aims to improve knowledge of the cells that initiate both beneficial immune responses against infectious organisms, and harmful immune responses against our own bodies- these cells are called dendritic cells (DCs). Understanding DC functions will enable us to design better vaccines, and treat inflammatory diseases.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This work will improve understanding of the way DCs control immune responses. These studies are likely to impact on the field of immunology generally. Collaboration with local clinicians will facilitate the transfer of any relevant insights from the animal studies to appropriate investigations involving clinical material, and may eventually lead to the design of better oral vaccines or treatments for inflammatory diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We anticipate that we will use 1400 rats and 5000 mice during the five years of the 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>We will collect lymph DCs from some live animals by cannulation of the thoracic lymph duct. This is a surgical technique; as such it will cause discomfort to the animals. This is minimised by the use of both local and systemic analgesics, under veterinary advice. Any animals showing signs of distress are humanely killed before suffering approaches carefully-defined limits. This is a procedure of no more than moderate severity.
The majority of the animals will be used either for collection of lymph DCs, as recipients of cells or molecules expected to modulate immune responses, or will be transgenic animals bred to be</td></tr>
</table>

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

	used in these ways. We do not expect any adverse effects, other than minor discomfort in most animals receiving treatments. Transgenic rats will develop arthritis and intestinal inflammation as they age, and some animals will be given substances that induce intestinal inflammation. These animals will be closely monitored. All animals will be humanely killed at the end, including any animal showing effects approaching the limit of the license.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studying the biology of DCs is difficult because they are extremely sensitive to their environment; their sensitivity to activation is integral to their function. This means that the characteristic features and functions of DCs grown in culture, or extracted from the tissues of patients or animals do not always correspond to DCs in their natural environment. However, the DCs we are able to harvest after surgery are collected on ice, seconds after leaving the animal. They are therefore extremely useful for investigations of DC biology. In parallel, and where appropriate, we will perform studies using DCs cultured from humanely-killed animals, or purified from human samples.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animals are treated in small groups, and the data generated from each animal are extensively analysed using appropriate statistical methods before deciding whether to repeat or modify an experiment. In this way the maximum information is obtained from each set of procedures, reducing animal use to a minimum.
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 work has to be conducted in mammals because the immune systems of invertebrates and non-mammalian vertebrates are very different. Rat and mice are the principal species used because they are the smallest animals in which thoracic duct cannulation is currently achievable. The rat has the disadvantages compared with mice that there are fewer reagents available and fewer transgenic and gene-targeted strains. However, rats will be used in some experiments because the rat model of inflammatory arthritis enables us to investigate a unique and important set of DC functions. Since beginning to work with these techniques we have significantly refined them. We have, for instance, developed new methods that remove the need to restrain rats or mice after cannulation. This process of refinement of our techniques will continue to evolve through the lifetime of this license.

Mechanisms of axon degeneration and its delay

Axon degeneration; Alzheimer's disease; motor neuron disease; peripheral neuropathy; ageing

- Summarise your project (1-2 sentences)

This project studies mechanisms of nerve degeneration as a model for axon loss in a range of neurodegenerative conditions including Alzheimer's disease, motor neuron disease and peripheral neuropathies. It also studies the loss of axons during normal ageing.

- 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.

Axons are 'wires' carrying signals from one nerve cell to another. They are essential for nervous system function and vulnerable to physical injury, toxins, viruses, metabolic defects and inherited disorders. This contributes to multiple sclerosis, motor neuron disease, Alzheimer's disease, glaucoma and peripheral neuropathies. There is no effective treatment for axon degeneration in any disorder.

We aim to identify methods to prevent axon degeneration. In 2001 we found a protein that delays axon degeneration and have built on this to identify drugs that have the same effect. Two main aims are to test their efficacy in a mouse model of an axonal disorder and to identify more effective ways to prevent axon degeneration.

We will study chemotherapy induced peripheral neuropathy (CIPN) because it is an excellent candidate for prophylactic treatment. CIPN is a lifelong chronic pain condition in many cancer survivors caused by their chemotherapy. It also limits chemotherapeutic dose, and hence the cancer therapy itself. As it involves only temporary axonal stress during chemotherapy at predictable times, CIPN is a model disorder for preventing axon degeneration. If successful, this knowledge could be applied to other axonal disorders.

The skills we have developed for studying axons put us in a unique position to study axon pathology in other disorders. We will investigate how the essential flow of molecules and organelles along axons is disrupted in Alzheimer's disease and motor neuron disease. This is important because the early stages of both disorders, when treatment has the best chance, involve massive loss of distal axons and synapses.

We lose a huge number of axons during normal ageing: 40% of nerve endings in our skin by 60 years and 45% of brain white matter by 80. This underlies the normal decline in mobility, memory, vision and other functions, and helps explain why ageing is the biggest risk factor for neurodegenerative disease. Initial studies suggest diet and exercise significantly alter age-related axon loss and we aim to understand the extent and timing of dietary intervention or exercise that are required.

- Outline the general project plan.

We will collaborate with a fly genetics group to identify genes that regulate axon degeneration flies and then validate their effects in mice, because confirmation in mammals is essential to know the full meaning of the data from flies. This combination of initial screening in flies and confirmation of the most important results in mice dramatically reduces the number of experiments needed in mice. We will then piece these steps

together into a molecular pathway, using cell culture methods where possible, to identify the best points to intervene using drugs. We will test the effect of one drug type, that we have already found to block axon degeneration, as a potential prophylactic method to prevent CIPN.

We will also study mechanisms of axon loss both in normal ageing and in mouse models of the age-related neurodegenerative disorders Alzheimer's disease and motor neuron disease. These studies will focus particularly on the decline in the delivery of essential molecules and organelles to nerves as we age and in many neurodegenerative diseases, a process known as axonal transport. Non-harmful transgenes allow us to image and quantify axonal transport in mice, a method that is impossible in humans. This approach also allows us to study what influences this axonal transport using whole nerves, thereby generating data that is much more physiologically relevant than in cell culture or in simpler species such as flies.

- 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.

Genetically altered mice with varying degrees of hindlimb paralysis will be used to model human neurodegenerative disorders. Nerve degeneration in mice or rats, induced surgically or by drugs injected into tissues, is important to identify factors that alter the course of degeneration. Mouse or rat models of CIPN will be used to test whether drugs can block the development of hypersensitivity to normally non-painful stimuli. Mouse models of obesity will be used to understand how this combines with normal ageing to cause axon loss.

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

Our work will advance scientific understanding of nerve degeneration and other cell death mechanisms. We are very likely to identify new genes that influence nerve degeneration and we also aim to translate this knowledge into novel drugs. In the long term this should benefit patients with a range of neurodegenerative conditions and in the short term we will promote public understanding of nervous system degeneration, and factors that cause it, through open access publishing and regular public engagement.

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

Most experiments will use mice (1-2000 per year) because their genome is well understood and models of the relevant human diseases have been established. A few will use rats (up to 100 per year) because some disorders are better modelled in rats than mice. Numbers will be minimised through careful use of pilot experiments, good experimental planning, use of cell culture methods and collaboration with groups using invertebrates.

- 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.

Replacement is used wherever possible, mostly through fly and cell culture studies, but confirming important results in a mammalian nervous system is essential. Without this, misunderstandings about disease mechanisms could eventually require more animals to

clarify. For ‘Reduction’ see section above and for ‘Refinement’ see below.

Human studies are important for confirming key animal data but cannot replace animals. Many human tissues are only obtained at disease endstage (death), human ageing studies are extremely slow and human genetic diversity and wide-ranging lifestyles complicate data interpretation.

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

Experiments are refined to minimise suffering using sterile conditions, anaesthetics, humane methods of killing, and targeting pathology to subsets of cells to avoid whole-animal suffering. The welfare of each animal is monitored daily by animal care staff, veterinary staff and/or scientists. If in rare circumstances an animal has an unexpectedly severe response to a drug or an operation, or where an infection develops, treatment is given where possible and if necessary the animal is humanely killed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Nutrition for sustainable cattle production</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cattle, nutrition, forage, environment</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[11]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>~~Yes~~</td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The objectives of this project are to advance knowledge and understanding of nutritional factors affecting cattle performance, metabolism, health and behaviour and to reduce their environmental impact.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">The project will advance the knowledge and understanding of feeding dairy and beef cattle. More accurate feeding will help avoid, diagnose or prevent disease or ill-health in cattle, and improve the production and quality of milk and meat. A greater knowledge of the nutrient requirements of cattle will assist in improving the welfare of animals by for example, better supplementation of cows grazing grass. This project will deliver greater knowledge on the quality, efficacy and safety of alternative forages and by product feeds (e.g. from bioethanol production) that are likely to become available as a consequence of climate change or energy production from crops. This project will also contribute towards protecting the environment through developing strategies to reduce the environmental impact of cattle through, for example, reducing methane production and excretion of nitrogen and minerals.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Cattle are the main species to be used, and the project will use approximately 400 dairy cows, 200 growing dairy or beef animals and up to 8 sheep.</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</td><td colspan="3">Most of the studies that will be conducted will be under conditions similar to that found on well managed commercial farms and commensurate with the code of practice, and will use forages or</td></tr>
</table>

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

level of severity? What will happen to the animals at the end?	supplements that are available to cattle farmers. To understand the effects of alternative forages and feeds, or dietary strategies on animal performance requires performance to be monitored. Milk yield will be recorded and samples taken for subsequent analysis in the same manner as on commercial dairy farms. Similarly, live weight and condition of animals will be measured in the same manner as on many cattle farms. To understand the underlying mechanisms of different forages and feeds requires samples of blood to be taken to determine metabolic status, samples from the rumen (the first stomach in a cow) to determine microbial metabolism, or the liver to determine aspects of metabolism. All of the procedures employed in this project are used in commercial practice by veterinary surgeons to monitor health in dairy and beef animals, except for the insertion of a permanent rumen fistula which is required to obtain rumen fluid for in vitro studies. To determine the effects of dietary treatments on aspects of fertility requires the reproductive cycle to be monitored by using, for example ultrasound. With any of these sampling techniques there is a risk of infection following the procedure, or pain or distress during the procedure. These will be minimised by the used of appropriate pain killers and by using trained personnel. The advice of a veterinary surgeon will be sought whenever necessary. At the end of the procedure the animals will be inspected by a veterinary surgeon and either returned to a farm or killed by a humane method (Schedule 1).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of this project is to improve the performance, health and welfare of cattle and to reduce their environmental impact. Because of the complex interactions between the diet and the cow, using cattle is the only scientifically viable option. This project does employ non-animal based alternatives (e.g. to measure the potential effects of different diets on methane output), and therefore reduces the use of animals. These techniques are, however limited and to determine whether they actually have a real effect in cattle requires them to be fed to animals and animal performance, health and metabolism monitored. Sheep have a similar rumen metabolism to cattle and will be re-used for rumen studies as they are easier to maintain and look after.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before a study commences the number of animals required is determined in consultation with a statistician. The number to be used is based on the literature and from similar studies in the subject area. Study design techniques such as change-

	over designs, where each animal receives each diet, or factorial designs are also employed to reduce the numbers and ensure that a significant difference can be detected.
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 use of cattle is necessary as there are no non-animal based substitutes that accurately replicate the combined effects of diet on intake, digestion, metabolism, milk production and growth. Additionally, as some of the dietary strategies may alter behaviour, the use of cattle is required. The animals will be kept, whenever possible under conditions similar to that encountered on well managed commercial farms and commensurate with the Code of Practice. For example, some studies will require the cows to graze at pasture, and others to be housed in stalls or on straw over the winter. Where samples are required, animals may need to be housed individually but will have sight and contact with others at all times. The use of appropriate pain killers will be used when required. If animals need to be restrained for example to measure urinary nitrogen output, they will have sight of other animals at all times and will be restrained for the minimum period to ensure that an accurate sample is obtained. The animals will be cared for at all times by trained and competent staff. All studies will be approved prior to commencing by a local ethics committee. This committee is made up of people with animal welfare, statistical design and animal health experience. The advice of a trained and experienced veterinary surgeon will also be sought at all times both in planning and during a study.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Fish Movements and Behaviour</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish, Movement, Behaviour, Telemetry, Otolith</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)[13]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td></td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</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>To gain a reliable and objective understanding of the movements, migrations, patterns of distribution and behaviour of marine fin-fish populations in relation to their environment, as well as discard survival, in order to provide advice in support of rational management and conservation to stakeholders, national and international governments and other international organisations.</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>Information on all aspects of fish migration and distribution in relation to the environment and discard survival will permit better advice to Defra policy customers and to the International Council for the Exploration of the Seas (ICES) on the rational conservation and management of marine fish stocks. The information will also contribute to the development of improved methods for assessing marine fish stocks. The work will also help provide a fundamental understanding of the relation between the movements, behaviour and distribution of fish, and in relation to their environment, thereby improving our capability to advise on the likely impacts of environmental change on fish stocks.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Fish, adults and juveniles. Approximately 1,775 animals will be used of the 5 year programme of work.</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</td><td>Most of the procedures are assessed as Moderate severity, some are Mild. Most animals will be involved in tagging studies and will be discharged from the Act and returned to the sea at the end of</td></tr>
</table>

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

level of severity? What will happen to the animals at the end?	the procedure. Possible adverse effect would be infection of tagging wounds. Risk of infection will be minimised by taking adequate antiseptic precautions during the procedures, by the topical application of a wide-spectrum antibiotic to sutures and tagging wounds, and by treatment with a systemic antibiotic (where appropriate).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim of the work is to use electronic telemetry methods (data storage tags or pop-up satellite archival tags) together with (where appropriate) otolith microchemistry and population genetics, to advance our understanding of the movements and behaviour of marine fish of commercial or conservation concern in relation to their environment. For this type of investigation there is no appropriate alternative to the use of conscious wild fish. In addition, the telemetry methods to be used are currently “state-of-the-art”.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental methods and numbers of animals used are based on previous experience and research. As part of Cefas’ Animal Welfare and Ethical Review Process, each programme of study is considered by staff from our in-house statistical team and their sign-off is required before any study is undertaken. The post-release survival of discarded fish in commercial fisheries will be investigated using electronic data storage tags, an approach that uses fewer fish than mark-recapture studies.
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 aim of the work is to advance our understanding of the movements and behaviour of marine fish of commercial or conservation concern in relation to their environment. Therefore a range of species including cod, eels, sea bass, spurdog, porbeagle shark etc. need to be studied. The methods chosen are based on previous experience and research that has been shown to provide evidence that is valuable in formulating advice to Government on factors that may affect fish populations and possible mitigation. Where fish undergo a procedure with recovery, they will be monitored for a suitable period in order to assess any adverse effects and ensure minimum suffering.

Sero-prevalence of Schmallenberg in sheep in the UK

- Summarise your project (1-2 sentences)

Schmallenberg virus is a very new disease of cattle and sheep which is spreading throughout Europe. It is known to cause considerable suffering to farm animals through very severe birth abnormalities in lambs and calves. Our study wishes to examine the spread of the disease within sheep flocks on 6 sheep farms in the UK.

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

We would like to study the spread of Schmallenberg virus on a small number of sheep farms to tell us whether the disease is present or not, how fast the disease is spreading in the flock and whether the farmer can expect problems at lambing time. Additionally, the data from these studies will be used by epidemiologists to develop models of disease spread which can be used in this country and abroad to develop improved disease control strategies.

- Outline the general project plan.

We wish to study the spread of the virus in six sheep flocks in the UK.
We will visit each farm every 3 months over a one year period and take a single blood sample from 40 randomly selected sheep on the farm. The sheep will remain with the flock and return to the care of the farmer.
The blood will then be tested for the presence of Schmallenberg virus in the laboratory.
Data will be analysed by veterinary epidemiologists.

- 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.

A blood sample will be collected, by an experienced veterinary surgeon from each sheep's jugular vein. It is expected the sheep will experience a transient mild discomfort from this. The sheep will remain on the farm with its flock at all times.

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

The information from this study will tell the farmer if his flock has the disease and whether to expect problems at lambing time. Also, the data from these studies will be used by epidemiologists to develop models of disease spread which can be used in this country and abroad to develop disease control strategies

- 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 have to use sheep as our animal species as sheep and cattle are the only farm animal species affected by this disease.
A statistician has calculated the minimum number of sheep we need to sample to obtain meaningful results from the study.

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

In order to study whether a farm has Schmallenberg virus or not and how quickly the disease is spreading in the flock we have to take a blood test from the sheep on the farm. We have used statistics to calculate the minimum number of sheep we can sample. The procedure is considered to cause only mild transient discomfort to the sheep.

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

  We have used the minimum number of sheep, the procedure of blood sampling is mild, it will be carried out be an experienced and licensed veterinary surgeon, the sheep will remain on the farm with their flocks.