Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries granted during 2013

Volume 42

Project Titles and key words

- Development of novel rodent control methods

  Non-anticoagulant, pheromone.

- Fat grafting with adipose stem cells

  Reconstruction, fat, adipose, stem cells

- Studies on Small Animal Models of Heart Disease

  heart failure atrial fibrillation hypertension

- Mitosis, Aneuploidy and Cancer

  Cancer, aneuploidy, mitosis

- Epithelium and Immune System interplay in the gut

  Intestine, immunity, infection, immune-regulation

- Metabolic phenotyping of rodents

- Cell division and differentiation in eggs and embryos

  Division differentiation Xenopus development

- Murine Model of Hepatitis B Virus Infection and Disease

  Hepatitis B virus, Pathogenesis, Genetic humanization

- Biocompatability Assessment of Medical Devices

- Poultry red mite control

  Poultry red mite, ectoparasite control

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Development of novel rodent control methods</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Non-anticoagulant, pheromone.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></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 research is to provide an alternative to anticoagulant rodenticides.

This research is in response to increased public demand for the control of diseases spread by rodents and a greater need to control populations with more humane methods.</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>Potential benefits of the project:

- Provides an alternative to exiting anticoagulant methods.
- A more humane form of control.
- Reduces the occurrence of anticoagulant resistance.
- Stops accidental dosing and death of non-pest species such as ducks, birds of prey and house hold pets.
- Protects the environment from toxic chemical build up.
- Reduces carbon emissions alongside a long lasting pest management processes.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Over a 5 year time period we propose to use approximately 500 rats and mice.</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>It is expected that the deployment of the rodent management method will result in a more humane and efficient form of control when compared with commercial rodenticides.</td></tr>
</table>

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

level of severity? What will happen to the animals at the end?	The rodent is expected to exhibit clinical signs of toxicity such as lethargy and reduced food and water intake as a result of the control method. The severity level will be classed as severe, as clinical signs of toxicity will be displayed as a result of the proposed control method. This classification is result of the protocols required to generate relevant data for the European Union in order to allow commercialisation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to allow a new rodent control method to be used commercially permission must be granted by the European Union. To achieve this, the Directive concerning this product type requires certain information about the products effect on the intended pest species. There is thus a legal requirement for these protocols to ensure the welfare of the animal is maintained when used commercially.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The majority of rodent control method investigations will be performed in glass (*in-vitro)* to reduce the number of rats and mice used under the project license. In order to reduce the number of animals further a preliminary protocol is employed to screen methods before more detailed evaluations. Specifically, this involves using animals one at a time. If the animals demonstrate undesired effects then the method will be abandoned and *in-vitro* investigations revisited. This prevents the use of ineffective control methods during data generation for the European Union.
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.	In order to ensure the quality and to minimise the number of animals used for the legally required data generation the European Union suggests specific guidelines. The research will then follow these guidelines to ensure that the data is acceptable to the European Union. The guidelines also state particular laboratory strains of rats and mice to perform the investigations on. This ensures that the laboratory data is suggestive of field use. Unlike many animal procedures, the species used in these experiments are the species which the control method is designed for, it is not a model for any other condition or system. Through out all the experiments the animals will be

	closely monitored for clinical signs of toxicity. This data will then be used to refine end points for future investigations and prevent any prolonged suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Fat grafting with adipose stem cells</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Reconstruction, fat, adipose, stem cells</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>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[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>To conduct fat grafting with human abdominal fat tissue primed with varying concentrations of selected stem cell subpopulations into an experimental mouse model and to measure the volume and survival of the grafted fat at increasing time-points up to 6 months. It is hoped that this will demonstrate the optimum stem cell concentration and subpopulation for fat graft survival.</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 project aims to be of benefit to several patient groups that require fat grafting procedures, usually in reconstruction after trauma or cancer. We hope to improve current reconstructive surgical practice with the addition of stem cell technology thereby avoiding non-efficacious procedures because current clinical methods of fat grafting usually result in recurrence of the volume deficiency and therefore require several repeat procedures. These studies will also be of wider benefit to those studying the mechanics of fat and of stem cells in regenerative medicine.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We expect to use up to 300 immunocompromised mice over 5 years.</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</td><td>We do not anticipate that this will affect the well-being of the animals. The procedure is of minor severity, and the animals should not show behavioural signs of pain nor effect on the animal’s</td></tr>
</table>

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

happen to the animals at the end?	mobility. Given that the animals are immune compromised, they will be monitored closely for evidence of infection. At the end of the study, the animals will be killed by a humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It would not be ethical to investigate the survival of fat graft substrate in humans because this project involves experimental stem cell techniques such as the selection of ASC subpopulations, which are not yet sufficiently established to justify clinical trials. Undertaking these studies on animals offer the most realistic alternative to obtain meaningful and comparable results in a relatively short period of time.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before the animal procedures are undertaken there are several laboratory experiments that are being undertaken in order to reduce the numbers of animals involved and to refine the parameters for the animal procedures. We have also calculated the minimum number of animals required in order to demonstrate a significant result in this 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.	Mice represent the lowest vertebrate group on which the parameters of fat graft are well characterised. This procedure will be carried out whilst the animals are fully anaesthetised. The animals should not have adverse effects from a short anaesthesia. Furthermore, the animals should not bear any ill effect from having fatty lumps on their back. Given that these animals will be immunodeficient, special attention will be paid to their well-being post-operatively and they will be observed closely for evidence of infections.

Studies on Small Animal Models of Heart Disease
heart failure atrial fibrillation hypertension

- Summarise your project (1-2 sentences)

This project is about how different forms of heart disease affect the electrical wiring system of the heart, which is called the cardiac conduction system (CCS).

- 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.

Heart failure patients may die suddenly, possibly because part of the CCS stops working. We need to study the effects of heart failure on the components of the CCS in order to find out why and how the heart's pacemaker is affected in this condition.
Pulmonary artery hypertension (PAH) is a severe condition found in babies and adults, with a high mortality. We want to find out why abnormalities of heart rhythm occur, and to what extent inflammation plays a part in this disease.
Atrial fibrillation is common and predisposes to stroke in man. We want to find out whether the CCS is affected in this condition, and if this may contribute to less effective contraction of the heart muscle.
Exercise training gives rise to a slow pulse rate. Although it is commonly thought that this is due to overactivity of the vagus nerve, we want to see if exercise can directly affect the CCS, and so predispose to an increased need for a pacemaker in later life.

- Outline the general project plan.

Animal models of different forms of heart disease are set up using different species as appropriate to the scientific questions. The effects of the disease process on the CCS are determined by different protocols as follows: 1. ECG recording and echocardiography on the intact animal; 2. electrical recording from pieces of tissue excised from the components of the CCS after sacrifice of the animals; 3. biochemical measurements of RNA from excised tissue; identification of proteins using antibodies which render them visible under the microscope, in excised tissue.

- 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.

Heart failure is induced by disruption of the valve between the main pumping chamber of the heart, by means of a tube through a neck artery, which is then removed; and at a second operation, a narrowing is placed around the main artery in the abdomen.
Atrial fibrillation is induced by implantation of a pacemaker. PAH is induced by a single injection of a plant substance called monocrotaline. Exercise training is done by supervised swimming in mice.

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

In heart failure parts of the CCS do not work properly, which can cause sudden death and requirement for pacemaker implantation in man. Once we know what happens to the proteins in the CCS it may be possible to stop or even to reverse these changes by gene therapy or drug treatment.
People with atrial fibrillation have impaired heart function, part of which may be due to disease of the CCS. Knowledge of how the CCS is damaged in this condition will enable treatment to improve heart function.

In PAH the aim is to prevent or reverse the condition, eventually by using designer drugs to interfere with inflammation; and to reduce the burden of abnormal heart rhythms and death. This will require further work that is beyond the scope of the current licence.

- 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.

Rabbits will be used, ~160, for heart failure and atrial fibrillation work because the hearts are closer to the human in the way the pumping is coordinated, compared with rodents.
For work involving the background to heart failure mice will be used, ~128, because transgenics will enable us to dissect out possible mechanisms of CCS protein alteration.
PAH will be studied in rats, ~64, because the disease can be induced by using just a single injection.
Exercise training will be done in mice so that work leading on from this, using transgenic animals, can be envisaged directly.
The numbers used are determined by calculating statistical “power”, which ensures that the changes observed are valid, and that when no change is apparent, this result is real and has been achieved using the minimum number of animals.

- 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.

Information about the effects of heart disease on the CCS can be obtained only by working with the living, intact heart, which means using live animal models. Human data are sparse and are almost always affected by drug treatment. We are extensively involved in computer modelling which helps with data interpretation and even in experimental design, but this cannot replace results obtained from animals.

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

Using 2 separate operations to induce heart failure reduces mortality from doing both operations at the same time, and the symptomatic burden is lower because the condition develops more slowly. PAH is induced by a single injection rather than repeated surgical procedures, which involve more trauma for the animal and a longer duration of the disease. The pacemaker generator that we will use for the atrial fibrillation experiments will be fully-implanted, rather than using an external pacemaker which is less well tolerated. For exercise training we are unable to ask the mice whether they prefer swimming to being put in a treadmill but with swimming the duration is shorter, and it is more easy to identify when a mouse becomes exhausted and needs to be removed.

Mitosis, Aneuploidy and Cancer

Cancer, aneuploidy, mitosis

- Summarise your project (1-2 sentences)

Normal human cells have 46 chromosomes. By contrast, cancer cells often have abnormal numbers of chromosomes. Our research aims to understand firstly how cancer cells accumulate abnormal numbers of chromosomes, and secondly how they survive having abnormal numbers of chromosomes.

- 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.

Normal human cells have 46 chromosomes, 22 pairs of autosomes and two sex chromosomes, females having two X chromosomes and males one X and one Y chromosome. By stark contract, human cancer cells often contain abnormal numbers of chromosomes. For example, the famous cancer cell line HeLa, has about 82 chromosomes. This deviation from the norm is known as "*aneuploidy*", see http://en.wikipedia.org/wiki/Aneuploidy for a more detailed description. In humans, aneuploidy has been associated with poor patient prognosis, more aggressive tumour growth, resistance to chemotherapeutics, and metastasis.

How does aneuploidy arise in the first place? Latest research suggests that aneuploidies are caused when cells divide, or more specifically during the chromosome segregation process that takes place just before cell division, i.e. during mitosis, see http://en.wikipedia.org/wiki/Mitosis. Normally mitotic chromosome segregation results in the chromosomes being pulled into two equal sets so that when the cell divides, each cell gets one complete set. However, if the chromosomes are not segregated accurately, the daughter cells could end up gaining or losing copies of particular chromosomes.

To guard against this problem and to thus ensure accurate chromosome segregation, human cells employ a surveillance mechanism – called the "*spindle checkpoint*", see http://en.wikipedia.org/wiki/Spindle_checkpoint – which monitors the segregation process and delays cell cycle progression until accuracy can be guaranteed. Unfortunately, this mechanism does occasionally fail. A striking example of this is Down's Syndrome which arises due to segregation errors in the germ line giving rise to individuals with an extra copy of chromosome 21, see http://en.wikipedia.org/wiki/Down's_syndrome. As mentioned above, cancer cells also have abnormal numbers of chromosomes and we now know that this is because they frequently make mistakes during mitosis.

Because this primary surveillance mechanism sometimes fails, cells have a backup mechanism; if a newly divided cell has the wrong number of chromosomes it initiates cell suicide in order to protect the organism as a whole. However, as a cell evolves from a normal cell into a tumour cell, not only does it make more segregation errors, but it also subverts this backup mechanism, thereby allowing aneuploid cells to continue to grow and divide. This then raises an interesting question: why can cancer cells tolerate aneuploidy whereas normal cells do not?

An interesting exception to this story is the liver. Whereas most cell types do not tolerate aneuploidies, it appears that like cancer cells, liver cells also seem to tolerate high levels of aneuploidy. Why this is so is unclear but it may allow adaptation to liver damage. This raises an interesting possibility; maybe tumour cells tolerate aneuploidy because they exploit the same tactic employed by liver cells. Therefore, if we can understand the differences between liver cells and other cell types with regard to aneuploidy tolerance, then we may be able to devise strategies that bolster aneuploidy intolerance in tumour cells. In turn, this may allow us to design therapeutic approaches that selectively kill

aneuploid tumour cells while sparing normal cells.

- Outline the general project plan.

We previously generated a strain of mice in which a spindle checkpoint gene can be inactivated at will. We showed that when this gene was inactivated in various cell types, aneuploidy occurred due to spindle checkpoint failure. This in turn resulted in activation of the backup mechanism which in turn caused cell death. However, liver cells survive inactivation of this gene, consistent with the notion that they can tolerate aneuploidy. To try to understand why liver cells can tolerate aneuploidy we plan to study this phenomenon in more detail. The project will consist of four stages. In stage 1 we will establish a colony of genetically engineered mice suitable for subsequent experiments. In stage 2 we will inject the mice with a harmless drug that will induce the inactivation of the spindle checkpoint gene in the liver. Our previously work shows that inactivation of this gene in dividing cells induces aneuploidy. Initially however, this in itself will have little effect as there are very few dividing cells in the adult liver. In stage 3 therefore we will inject the mice with carbon tetrachloride (CCl_4) in order to specifically induce acute liver damage. This will cause the non-damaged liver cells to proliferate in order to repair the damage. In stage 4 we will humanely kill the mice in order to isolate their tissues for analysis.

- 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.

Stage 1 will involve standard animal husbandry techniques and as such will involve very little by way of harm. The injections in stage 2 and 3 will be carried out by qualified animal technicians, so any pain will be mild and temporary, so again, predicated harms are minimal. The carbon tetrachloride method has been selected specifically because it induces acute damage as opposed to liver failure. This is a well established technique which damages hepatocytes surrounding the central vein, triggering the adjacent hepatocytes to divide in order to replace the damage. Regeneration is normally complete within 7 days and as such, the overall severity of the procedure is mild. Note that the gene inactivation method in stage 2 will also affect the intestine. However, because gene inactivation is not 100% efficient, the unaffected cells rapidly repopulate the tissue so that within several days it is back to normal, and as such any adverse affects will be minimal. Overall therefore the predicted harms are considered to be mild.

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

With regard to potential benefits, it is clear that the majority of human tumours are aneuploid, not only because they make errors during mitotic chromosome segregation, but because they also then tolerate those errors. One of the holy grails of modern cancer research is to try to exploit the differences between tumour and normal cells to identify strategies which selectively kill tumour cells. However, at present there are no therapeutic strategies which exploit the fact that tumour cells are aneuploid. This is simply because we do not yet understand enough about (a) how the errors arise in the first place and (b) the mechanisms that normally induce cell death when aneuploidy arises. Our hypothesis is that tumour cells tolerate aneuploidy because they exploit a normal tactic that is switched off in most cell types such as fibroblasts and epithelial cells, but remains active in liver cells. If we can delineate the differences between liver cells and epithelial cells with regard to aneuploidy tolerance then we may be able to gain some insight into how to bolster aneuploidy intolerance in tumour cells.

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

The genetically engineered mouse is the *de facto* standard system for modelling human disease *in vivo* due to the high degree of genetic conservation between humans and mice, coupled with the experimental tractability of genetic engineering in the mouse. In addition to these well established advantages, using mice will allow us to build upon a pre-existing body of knowledge and reagents. Indeed, we will use a strain of mice that is already established and well characterised, and we will follow up a series of previous experiments that indicate our approach is likely to yield informative results. In the first instance we will require about 200 mice per year in stage 1 in order to generate ~40 animals of the right genotype suitable for stage 2, so that we can then take on ~20 to stages 3 & 4. If all goes well the project will take ~2 years thus requiring a total of ~400 mice. We will use both males and females for stages 2-4 in order to maximise the efficiency of the colony. We have opted for a CCl_4 injection rather than partial hepatectomy because the latter has the potential to introduce more variability which in turn would require more animals.

- 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.

With regard to replacement, while we also use cell lines to study the causes and consequences of aneuploidy, our understanding of these processes in physiologically relevant contexts is limited. Therefore, using model systems that allow mitosis to be manipulated in an intact tissue are imperative. In this context, the mouse has become the *de facto* standard system - see above.

With regard to reduction, keeping animal numbers to a minimum will be facilitated by the fact that the genetically engineered strains necessary for this program of work have already been established, plus the breeding and genotyping regimens are routine. Consequently, we will only need to establish a small colony. Secondly, the experimental system has already been set up and established. Moreover, the liver regeneration protocol is tried and tested and will thus require very little optimization.

With regard to refinement and minimising animal suffering, note that the predominant activity will be routine animal husbandry. Gene inactivation and triggering liver regeneration will be mediated via simple injection. We have opted for a CCl_4 injection rather than partial hepatectomy because the latter requires surgery and thus has the potential for more suffering.

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

The protocols involve breeding and maintaining a colony of mice followed by a series of simple drug administrations to induce genetic alterations and trigger liver regeneration. The mice will then be humanely killed for downstream analysis. *In toto* these procedures will involve very little suffering.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Epithelium and immune system interplay in the gut</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Intestine, immunity, infection, immune-regulation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</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>
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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[6]</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>Aim of this project is to dissect in details the interaction between the gut epithelium and the underlying immune system.
Although in the past few years our knowledge on these interaction has improved, the key events at both cellular and molecular levels still remains to be determined.</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 project will gather information that will advance knowledge in important areas such as i) oral delivery of vaccines to improve immune defences at locations most vulnerable to infections, ii) understanding how the body defends itself from pathogens and ultimately iii) provide new information on intestinal mechanisms that play a role in the onset of allergic reaction to food</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>We have chosen mice as our experimental model.. We expect to use a total of 2900 mice over 5 year period. This number includes breeding of genetically modified mice that will express specific markers for a particular type of immune cells.</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 severity of the procedures we intend to use ranges between mild to moderate; these mainly encompass the delivery of microbes and food allergens via the oral route. After each procedure mice will be monitored to evaluate possible side effects, such as significant weight loss, hunching, crouching or piloerection. At the end of the</td></tr>
</table>

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

	procedure mice will be killed according to established methods
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, the extreme complexity and sophistication of the immune system cannot be featured by using in vitro system and the use of whole organism is required to generate and study the very many components (both cells and molecules) of the immune system and their role in the regulation of the immunity. In our laboratory, we are currently using human specimen (biopsies) from the local hospital to establish in vitro organ culture using human biopsies. However, the intrinsic difficulties in maintaining the intestinal tissue viable in culture for long time prevent us for using this approach for a variety of experiments. Also, in vitro systems are being exploited. We are thus well set to seek all possible types of replacement for animal research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This whole project has been designed with the idea of using the minimum number of mice required to acquire meaningful scientific information. This is done by evaluating the different types of experimental procedure to be performed, our previous experience and data already in the literature. This approach enables us to perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative. Also in a wider context, to maximize the information from a single animal, we aim to collect samples from multiple body sites and provide those samples to appropriate scientific colleagues.
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 main reason behind the selection of mice is in the fact that the mouse is the most used model to study the immune system. This is due to the similarities between the human and murine immune system, the extensive knowledge of the murine immune system and the large variety of reagents available to identify and study regulatory pathways. Also, mice can be genetically manipulated in order to assess the function of specific molecules of interest to the project. Wherever necessary we shall use local and general anaesthesia to avoid animal suffering. In all long term experiments animals will be killed if they show any sign of ill health during experiments such as hunching**,** significant weight loss, hunching, crouching or piloerection and breathing difficulties.

Project Title (max. 50 characters)	**Metabolic phenotyping of rodents**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals[7]

[7] At least one additional purpose must be selected with this option.

Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In the last 20 years obesity has increased to epidemic proportions. In Britain a third of the adult population are considered obese. Obesity increases mortality because of associated diseases such as diabetes, coronary heart disease and an increased risk of cancer. There is a clear unmet clinical need in the treatment of obesity; only one therapy is currently available, which is only mildly effective whilst having undesirable side effects. The objective of this Project License is to provide information on the complex interactions responsible for the regulation of food intake and energy balance that occur between the gastrointestinal tract and the brain. Increasing our knowledge of how body weight is regulated may lead to the discovery of new medicines which can be used to treat diseases such as obesity, anorexia nervosa and eating disturbances associated with cancer. We will firstly investigate the effects of acute administration of both centrally and peripherally administered substances (e.g. neuropeptides, neuromodulators, circulating factors, synthetic analogues, antagonists, neutralising antibodies, metabolites, ions and proteins) on a number of endpoints. The CLAMS system will facilitate the 24 h non-invasive monitoring of oxygen consumption and carbon dioxide output, whilst providing accurate activity and food intake measurements. Implantation of telemetry probes will facilitate the monitoring of blood pressure and temperature. The effect of substances on plasma glucose and insulin levels, as an indication of diabetic state, will also be investigated. Some substances will be administered chronically either centrally or peripherally to investigate the effects of long-term administration on these endpoints. Mice with targeted deletions or targeted over-expression of hypothalamic neuropeptides and circulating factors will also be used for these studies. This project will lead to a greater understanding of the control of appetite regulation and energy balance. This knowledge is necessary to identify new medicines which are needed for the treatment of obesity which will provide an improved quality of life for those people who suffer from this debilitating and life threatening disease.

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)?	Obesity, a major medical problem in Western society, results from a long-term disturbance of energy balance. Currently it is estimated that there are over 1 billion people overweight. It is estimated that in the UK, obesity causes 30,000 deaths a year. Studying the interactions of hypothalamic neuropeptides with gastrointestinal factors in the regulation of energy balance will aid our understanding of the patho-physiology of obesity and metabolism, with a view to the development of effective agents in the treatment of obesity
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice were chosen as the systems that regulate energy homeostasis are almost identical to those in humans making these the ideal model organism to use. The number of animals used in individual studies has been worked out in consultation with a statistician to keep the animals used to a minimum; we anticipate we will use significantly less than 4000 rats and 3000 mice year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The adverse effects will depend upon which of the protocols the animal undergoes. Adverse effects expected are motor control and co-ordination disruption, excessive weight loss or inhibition of food intake mild dehydration minor anorexia and minor weight loss. The expected level of severity is moderate. At the end of the experiemnts all animals will be killed either by a schedule 1 method or by exsanguination under terminal anaesthesia, or by decapitation

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	When possible and appropriate substances will be tested in vitro to initially characterise them. Also ex vivo approaches such as release of neuropeptides from hypothlamic organotype cultures will be used where possible. However, the systems to be studied are complex physiological processes that are dependent upon the interaction of several separate systems within both the CNS and the periphery. In vitro assays cannot adequately model the complex physiological systems involved in energy homeostasis, therefore further in vivo work will be required in some cases.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure that the numbers of animals used are kept to a minimum, all orders for animals and experiments involving the use of animals require explanation and discussion before they can be authorised by myself or by a designated senior member of the laboratory. All of the experiments will be planned using power calculations to ensure the minimum number of animals required to achieve statistical significance are used for them, an example of which is given below: In experiments investigating the long term effects of altered arcuate glucokinase activity on food intake and the effect of glucose feeding on food intake require group sizes of fifteen animals. The size of the groups was calculated using the computer programme G*power. Power analysis was conducted for an F test repeated measures analysis. The power analysis was performed on the expected change on food intake using data from our pilot studies. The study is powered to detect a 100g difference in cumulative food intake (10%) with a standard deviation of 100 at the 0.05 level giving a required group size of 15. Where practicable multiple end point analysis will be performed on the tissues obtained from animals at the end of the studies to minimise the number of groups of animals required.

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 two species used in this work will be mice and rats. Together these are the most widely used model organisms for studying metabolic parameters. The main reason for this is that the systems that regulate these functions appear to be conserved between rodents and humans in many instances. For example many of the established hypothalamic regulators of energy homeostasis, such as leptin, are known to have the same function in both rodents and humans. Suffering of the animals will be minimised by the appropriate use of anaesthesia and analgesia to control pain. We will also utilise techniques which minimise the suffering, for example we have recently developed a novel methodology for conducting glucose tolerance tests, where glucose is administered by free consumption rather than gavage or intra-peritoneal injection.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Cell division and differentiation in eggs and embryos</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Division differentiation Xenopus development</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)[8]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[9]</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">The main scientific objective of this project is to characterise mechanisms regulating the control of cell division and adoption of specialised fates in cells and tissues in developing Xenopus embryos in vitro and/or in vivo.
We also aim to manipulate proliferation and cell fate using Xenopus egg extracts.</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">Advances in fundamental knowledge will benefit the developmental biology community who strive to understand these crucial events in development. Moreover, the knowledge advances gained under this licence can be applied to mammalian cells, and may then be exploited (in mammalian systems) to improve therapeutic approaches in diseases of perturbed division and cell function such as cancer. Furthermore, manipulation of these processes might be useful in regeneration e.g. generating islets in culture for treatment of diabetes, neurons in culture for treatment of Parkinsons disease. These longer-term benefits will be realised in 5-10 years and will fall outside the scope of this Project Licence.</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">Xenopus frogs, approx. 840 over 5 years.</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">The injection procedure is very mild and adverse reactions very rare. Rare infection around the site of injection or retention of eggs in the body cavity leading to bloating and illness occasionally occur,</td></tr>
</table>

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

level of severity? What will happen to the animals at the end?	and would result in Schedule 1 killing. Female frogs will be subject to reuse, reinjecting for ovulation no more often than every 3 months. Reuse of males after a suitable rest period may occur, where hormones are injected to promote sperm maturation prior to natural mating. Re-use will minimise the numbers of frogs used and the procedures are very mild with negligible adverse effects. When egg laying is no longer reliable, Schedule 1 killing is undertaken. Continued use of animals may occur when we are using genetically modified frogs that have been genotyped to generate eggs and sperm.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Embryo development occurs through a complex network of genetic and cellular interactions that is largely unknown, and the nature of the response of a cell to a specific signal depends upon both the tissue environment and the previous developmental history of that cell. Since these cannot be reproduced in *in vitro* systems, and the purpose of our studies is to reveal how the nervous system and endocrine pancreas (among other tissues) develop, it is currently only possible to uncover the underlying mechanisms by studying gene function and cell behaviour in the context of embryo development. In addition, frog eggs have dramatic genetic reprogramming abilities, which cannot be recapitulated using purified proteins.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Frogs must be ovulated to generate eggs for all aspects of these experiments. We will only ovulate the minimum number of frogs to provide the required number of eggs/embryos. Frogs will be reused for ovulation and natural mating after an appropriate rest time to minimise the number of animals to be used. Occasional reuse by other licenced projects may occur with healthy frogs to minimise 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.	Xenopus have been selected because, after hormonal stimulation, they produce numerous eggs that can be used to study protein biochemical modification in response to e.g. cell cycle transitions, and after in vitro fertilisation or natural matings, will produce embryos ex vivo that are large enough for biochemical and embryological manipulation. Individual animals are re-ovulated after a suitable rest period and kept in groups in environmentally-enriched tanks

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Murine Model of Hepatitis B Virus Infection and Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Hepatitis B virus, Pathogenesis, Genetic humanization</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[10]</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[11]</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>Study of hepatitis B virus is currently hampered by the unavailability of suitable small animal model systems to study curative therapies and develop novel treatment strategies. The aims of this project are to identify host factors important for HBV infection and translate these results into a small-animal model recapitulating HBV-associated disease pathogenesis. Initially, we will identify host factors in murine cell lines and test their validity to confer permissiveness to HBV in mice using an adeno-associated virus-based in vivo delivery system. Ultimately, we will generate transgenic mice expressing all essential human host factors to create a mouse model for hepatitis B virus research. To validate this model, we will confirm all results obtained with human patient data available through the Section of Hepatology at the Imperial College London and furthermore utilize the current gold standard for HBV in vivo experimentation, human liver-chimeric mice, which harbour human hepatocytes and are permissive to HBV infection.</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 generation of a fully permissive mouse model for HBV would open the door to developing novel treatment strategies aimed towards curing infection. Despite the availability of a protective vaccine over 300 million people are currently chronically infected and over 4 million acute HBV infections are recoded annually. Treatment for HBV infection is merely suppressing infection and no cure is available.</td></tr>
<tr><td>What species and</td><td>The only species used in this proposal are mice. For the successful creation of an HBV mouse</td></tr>
</table>

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

approximate numbers of animals do you expect to use over what period of time?	model, ensuring statistical significance of results generated, a total of 1.600 mice were calculated over a total time-period of 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?	The proposed project consists of seven sub-protocols ranging from breeding and generation of transgenic mice (both mild), infection of mice with adeno-associated virus vectors, hepatitis B virus isolates and GM HBV, treatment of mice with HBV inhibitors licensed for human use to generation of xenotransplantation models modelling a human liver (all four protocols moderate). The final protocol consists of terminal anaesthesia and is thus rated as non-recovery. Most of the anticipated adverse effects will contain potential liver injury, either due to infection with HBV or due to the model background for the generation of human liver-chimeric mice. These effects may include among others listed under each protocol section piloerection, weight loss, reduced food/water intake, and subdued behaviour. Each experiment falling under one of the here proposed project will be closely monitored by using a clinical scoring system (attached to the application) to ensure severity limits are not exceeded. These limits are clearly defined in each protocol section and clearly defined in the clinical scoring sheet. Animals either reaching the severity limit or animals reaching the end of one of the suggested experimental protocols will either be killed under schedule 1 or will be transferred to protocol 19b.7 (Terminal anaesthesia without recovery). A special focus is given to liver-chimeric mice since their creation involves surgical injection (intrasplenic) of human cells followed by repeated induction of mouse liver injury to allow human liver cells to proliferate. The severity limit is set to moderate for this protocol since animals will be i) subject to post-operative analgesia and surgery will be performed minimally invasive to ensure complete and fast recovery and ii) animals will be closely monitored during the induction of mouse liver damage including clinical scoring. Each animal will be immediately subjected to analgesic treatment if signs of distress or pain are visible and will be killed under schedule 1 or transferred to 19b.7 (Terminal anaesthesia without recovery) should the severity limits be reached.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Physiological and pharmacological studies related to HBV are hampered by the absence of a realistic model of the disease. In vitro cell culture experiments have confirmed that two recently described molecules are indeed receptor

	candidates. At this stage of model development it is utmost necessary to investigate if this also holds true in vivo. Due to ethical constraints, inhibitors and treatment candidates based on epigenetic regulatory mechanisms cannot be tested in humans and hence mouse models provide the much-needed in vivo models for studying HBV-induced disease and therapeutic potential of epigenetic regulators of interferon stimulated gene induction. Although we would continue to employ in vitro cell lines and primary cells, yet they do not adequately model the complete array of molecular, cellular, physiological and behavioural interactions necessary to fully understand HBV.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals required demonstrating differences between groups with sufficient power will be used. For inhibitor testing, 4-8 animals will be tested in each group to show approximately 2 Log difference in the HBV DNA load. In order to limit the use of mice, experiments will be planned to combine the utilization of control groups, i.e. when testing the impact of two distinct treatments, experimental initiation can be planned to utilize one instead of two identical control groups, thus limiting the overall use of animals. Additionally, all animal group sizes have been calculated to obtain the highest degree of statistical significance while still limiting the number of animals in each experiment.
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.	Only mice will be used in the studies here. They represent the lowest vertebrates with a considerable degree of physiological and genetic homology with humans. The aim will be to ensure experimental outcome can be satisfied in the minimum possible time using the minimum number of animals, thereby reducing the suffering of the animals. Where possible, oral gavage may be used instead of intraperitoneal injections. This is considered as a method of refinement as in some cases oral gavage is less invasive compared to intraperitoneal injection and does prevent problems with possible irritation by compounds given by the i.p route. However, inhibitor availability would dictate if oral gavage could be used. Adverse effects will be prevented by close monitoring of the animals during administration of the anaesthetic and throughout the study/research. To reduce severity, animals will be monitored through the experiments. Animals may be comforted with fluid and warmth.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Biocompatability Assessment of Medical Devices</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Biocompatability, Medical Devices</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)[12]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[13]</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 this project is to determine local tolerance, sensitisation potential, systemic toxicity, intracutaneous reactivity, effects of implantation, mutagenicity and pyrogenicity potential of Medical Devices and biomaterials in order to ensure the safety of these products when in use with the human population.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>In the normal course of biomedical applications, biomaterials or medical devices can be in direct or indirect contact with patients. The potential hazards of the biomaterial or device to the patient depends upon the conditions and duration of contact between patient and device. There is an obligation on the part of the manufacturers to establish the safety of their products before they are marketed. Medical device safety evaluation assesses the risk of adverse health effects due to normal or exaggerated use of a device. Since adverse health effects could result from exposure to the materials from which the device is made, preclinical assessment of the toxic potential of such materials or components is needed to identify any potential hazards and to minimise risks to the patient. Wherever possible this information will be obtained using knowledge on the composition of the device or from in vitro methods, however the use of animal studies is essential in some circumstances in order to ensure the safety of a medical device prior to use in humans. The safety information will also allow appropriate Competent Authorities or Notified Bodies to make sound regulatory decisions on the risks of adverse health effects due to normal use, or possible misuse, in humans.</td></tr>
</table>

[12] Delete Yes or No as appropriate.
[13] 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?	During the lifetime of the licence the following numbers of animals are expected to be used. Mice: 2050 Rats: 850 Rabbits: 660 The majority of animals will be used in short term studies lasting up to 14 days.
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 majority of studies are involved with the investigation of local tolerance effects and the level of severity is expected to be mild. Signs of toxicity are not expected. Transient mild stress may be caused due to handling, restraint, application and removal of test item. Rabbits may be kept alive at the establishment for re-use in pyrogen tests (after veterinary assessment to ensure that their general health has returned to normal after any previous procedure) where this is authorised or will be humanely killed using a schedule 1 method. All rats and mice will be humanely killed at the end of the experiment 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	There are currently no validated alternative methods for the proposed animal studies. Relevant *in vitro* methodologies will be introduced as soon as the methods are validated for use with Medical Devices and the test guidelines are updated.
2. Reduction Explain how you will assure the use of minimum numbers of animals	*In vitro* screening methods will be used wherever appropriate in order to reduce the number of animals used in *in vivo* studies. Range finding studies will only be used when insufficient toxicity data is available to allow a main study to be performed. The re-use of rabbits in pyrogen testing, where they have not suffered any more than mild adverse effects from another procedure, minimises the number of animals used, and reduces overall stress to individuals by using those acclimatised to handling, without compromising the welfare of the individual animals concerned.
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 species is chosen to provide the maximum information and is consistent with the test guidelines. Humane endpoints will be specified in order to minimise or alleviate pain and/or distress consistent with the scientific objectives. Refinement is also achieved by systems of care and accommodation that enhance the animals welfare. Environment enrichment is provided wherever appropriate and group housing of social animals is encouraged unless precluded on scientific grounds.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Poultry red mite control</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Poultry red mite, ectoparasite control</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[14]</td><td>
<table>
<tr><td>Basic research</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></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[15]</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>Dermanyssus gallinae, the poultry red mite causes considerable health and welfare problems in most poultry species as well as in cage birds and wild birds. In European poultry production systems, D. gallinae is an important ecto-parasite of chickens, especially laying hens. Infestation with these mites causes serious reduction in bird performance, the development of a potentially fatal anaemia and an increased incidence of aggressive pecking and cannibalism, which can be a serious welfare consideration. The prevalence of poultry red mite is very high with 60-90 % of laying hen units affected in the UK.

Infestations by the poultry red mite are more prevalent in alternative poultry systems and, in the EU in particular, there is an increased consumer-driven movement away from cage-egg production systems to more extensive alternative systems, and therefore the control of poultry red mite is becoming increasingly important. In addition EU legislation (EC directive 99/74) requires that cages used in egg production contain specified furniture (eg scratching area, perches, nest box etc) which in themselves have the potential to provide extra harbourages for the red mite.

Currently, the main method of control of the poultry red mite is to use a combination of house sanitisation (vacuuming, steam cleaning) followed by spraying with a variety of acaricides. However, the control of poultry red mite has proved very difficult to manage due to a combination of physical</td></tr>
</table>

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

	and chemical factors. The laying hen is typically housed in cage or aviary type systems which contain a considerable amount of “furniture”, such as perches, feeding troughs, nest boxes which are typically constructed from wood or metal with unsealed edges. The poultry red mites feed on hens during the night and retreat to these crevices during the day and are therefore difficult to physically dislodge from the poultry house. With either concern and/or the removal of organo-phosphate based acaricides there is a need to identify equally efficacious compounds for the control of the poultry red mite. We have developed an in vitro screening test that is being used to screen a wide range of novel potential acaricides, including biological agents. This license is requested to enable compounds that have passed the in vitro screening phase to be tested in vivo (ie red mite will be added into the environment in which poultry are housed and a range of acaricide-type compounds tested for efficacy against the red mite). The nature of the parasite and its life cycle, combined with the laying house environment in which acaricides are used is such that in vitro testing does not enable a complete evaluation of product efficacy.
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 intend testing a range of acaricide-type compounds which, though in vitro testing, have shown themselves to be effective against the poultry red mite. While in themselves these studies will not enable products to be brought to the market, they will enable the range of compounds being evaluated to be refined and have the potential to make a considerable contribution to the health and welfare of laying hens world-wide.
What species and approximate numbers of animals do you expect to use over what period of time?	*Gallus gallus domesticus*; up to 400; over 5 five 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?	At worst we would expect adverse effects – primarily irritation to the skin - to be transitory and no more extreme than mild. *At the end of the study the majority of animals will be discharged from the controls of the Act and released ‘to stock’.*
Application of the 3Rs	
1. Replacement State why you need to use	In vitro tests are used extensively in the project but there are some elements of efficacy that can only

animals and why you cannot use non-animal alternatives	be tested in vivo and more specifically on the target host, in an environment that simulates the commercial laying house. Factors that cannot be tested in vitro adequately might, depending on the mode of action, include resistance of the product to degradation by the chemical (eg bird faeces and their breakdown products) or the physical (eg dust, bird activity, faeces, humidity) environment in the laying house; the ability of the compound to either penetrate or be carried into the mite harbourages.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments will be reviewed by SRUC's Animal Experiments Committee and part of this review process involves scrutiny, by the Committee, which includes a professional statistician, of the 3Rs and in particular that the project team has aimed to minimise animals used in studies to a number compatible with the aims 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.	As outlined above in the section covering 'Replacement' the laying hen represents the animal of choice for this particular project, in part because of the life cycle of the Red Mite and the way in which the host and parasite interact with their environment. This interaction cannot be adequately modelled by the use of other species of animal. Bird welfare and health will be monitored closely and any bird judged to be in ill-health will be removed from the study (ie it is not the purpose of the project that birds become ill as a result of the red mite challenge or the control measures).