# Animals (Scientific Procedures) Act 1986

## Non-technical summaries for projects granted during 2014

## Volume 15

Projects with a primary purpose of Basic Research into the Endocrine System/Metabolism

**Project titles and keywords**

1. **Regulation of pituitary physiology**
    - Pituitary, gene regulation, prolactin

2. **Genetics of Metabolism**
    - Diabetes, obesity, diabetic complications, ageing

3. **The aetiology of metabolic disorders**
    - Diabetes, obesity, insulin secretion, channelopathy

4. **Islet pretreatment to reduce hypoxic graft damage**
    - Diabetes, islets, transplantation, hypoxia

5. **Oral delivery of test agents**
    - Oral gavage, diabetes therapy

6. **Neuroplasticity, immunity and behaviour**
    - rodent, seasonality, leukocytes, reproduction, neuroangiogenesis

7. **Metabolic alterations of pregnancy**
    - Pregnancy, metabolic disease, offspring, therapies

8. **Modulation of endocrine pathways by imprinted genes**
    - Imprinting, hormones, development, epigenetics, metabolic.

9. **Relationship between diabetes and cancer**
    - Diabetes, Cancer, Pancreatitis

10. **Energy Homeostasis and Metabolism**
    - Food intake, energy expenditure, obesity

11. **Effects of mitochondrial functioning on mammal phenotype**
    - Endothermy, BAT, oxidative stress, rodents

12. **Nuclear envelope roles in health and disease**
    - nuclear envelope, genorne organ isation, metabolism 5 years

13. **The role of copper in diabetes and ageing**
    - arteries, kidney, heart, eyes, biomarkers, treatment

14. **The Role of NPPC in Pituitary Development**

- C-type natriuretic peptide (CNP) Genetic modification Tissue specific, Mouse

**15. Genetic Toxicology – Chemicals**

- Mutagen, Genetic Toxicology

**16. Impact of gut microbiota on type II diabetes**

- Microbiota, Metabolism, Type II diabetes, Obesity

| **PROJECT 1** | **Regulation of pituitary physiology** | |
|---|---|---|
| Key Words (max. 5 words) | Pituitary, gene regulation, prolactin | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3) | 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 |
| | X | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | The pituitary gland produces hormones that control a wide range of body functions. Hormone production is regulated both acutely in response to immediate stimuli such as stress and trauma, and also chronically in response to physiological changes like puberty and pregnancy. It has been found that gene expression in the pituitary is dynamically variable, both in tumour cell lines in vitro, and in normal animal tissue. We are exploring the mechanisms and significance of the dynamic responses of gene transcription in living cells, and how this relates to pituitary growth and development. An important aspect of this work is the ability to study normal pituitary tissue, as opposed to cancer cell lines.<br><br>Transgenic rats are available in which marker genes are expressed in the pituitary gland. These marker genes have no detrimental effect on the pituitary gland itself or on the animal's well being, but allow us to study the behaviour of pituitary tissue that has been removed from the animal after death. | |
| What are the potential benefits likely to derive from this | The work will lead to new understanding of the role of tissue structure in coordinating the production of | |

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| project (how science could be advanced or humans or animals could benefit from the project)? | hormones in normal physiology. This in turn will help us understand how tumours arising in the pituitary gland over-produce these hormones, and we hope it will contribute to the development and understanding of new therapies for these tumours in man. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Rats and mice, up to 4000 over 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? | Very few if any adverse effects are expected. Most of our studies will be aimed at normal physiology in healthy animals, for example during early development, pregnancy and across the oestrous cycle. In some experiments we will study the effect of oestrogen, using hormone implants similar to those used by women in routine healthcare. Discomfort from oestrogen implant administration will be minimised with analgesia. In rats, oestrogen administration can cause weight loss, and weight will be carefully monitored, and experiments terminated if weight loss exceeds 15% - in most cases the severity limit is likely to be mild, with a maximum severity limit of moderate. Animals will be humanely killed at the end of experiments. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | The aim of the work is to study effects of tissue structure on hormone production. Cancer cell lines have been studied until now, and limited work can be performed in this way on individual cells, but for analysis of tissue<br><br>structure animal work is essential. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | We are using microscopic approaches studying the behaviour of individual cells, and this allows us to minimise the numbers of animals to ensure reproducibility of results consistent with achievements of scientific goals. |
| **3. Refinement**<br><br>Explain the choice of species | We are using laboratory rodents as these are amenable to genetic manipulation, and the physiology |

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| 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. | of the pituitary gland is well understood.<br><br>Animals are observed at least once a day. No adverse effects are expected from the endocrine manipulations. Analgesics will be administered after surgical procedures to minimise discomfort or pain, and we will seek veterinary advice if we encounter any unexpected occurrences. |

<table>
<tr><td>PROJECT 2</td><td>Genetics of Metabolism</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Diabetes, obesity, diabetic complications, ageing</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)</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</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We aim to understand the genetics and physiology of metabolism with a particular focus on diabetes and obesity. Our objective is to identify new genes in this process and understand how genes implicated in human disease function normally and in the disease state. Our objective is to better understand the basic biology, functionally validate novel genes and identify potential therapeutic targets</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>We will develop new models for investigating diabetes and advance basic science by understanding the role of particular genes in disease. If some of our targets appear to be tractable we will seek to engage in the first steps in the drug discovery process so that our results can be translated for the benefit of the patient.</td></tr>
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| What species and approximate numbers of animals do you expect to use over what period of time? | Mouse, 73,000 over 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? | Mice with overt diabetes may loose condition over time. Metabolic phenotyping procedures are generally well tolerated and we use a local anaesthetic in blood sampling to minimise any pain. Where appropriate we use general anaesthetics and limit the frequency of these. Some techniques require individual housing during the procedure, which may be stressful. The maximum expected level of severity is moderate. At the end of the experiment mice will be humanely killed. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Diabetes and obesity is a complex disease and involves the interaction of many organs in the body that communicate through hormones, metabolites and the nervous system. It is not possible to replicate this complex system *in vitro*. However, where possible we do use *in vitro* techniques to investigate specific aspects of the disease as appropriate. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | We use statistical methods to analyse our results and to calculate the numbers that we require for an experiment in order to be able to observe a trait and generate robust and accurate data. These calculations are based on experience with different techniques. We have access to a trained statistician who is able to help us achieve this and to develop better approaches. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs | The mouse is a good model for diabetes and obesity and shares many similarities and physiological traits with humans. Although control of blood glucose metabolism and body composition is very highly conserved across many species and there are advantages in looking at lower species (like fish or flies) as appropriate in some specific experiments the mouse remains the most refined model for comparison with human systems as it |

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| (harms) to the animals. | reflects the best physiological match. We will minimise harm to animals by applying the highest possible welfare management and monitoring so that problems are picked up and treated quickly and inform future practice. We will use anaesthetic when pain is likely to occur, for example in a procedure such as blood sampling. We will collect data by the least invasive and most efficient route. We will anticipate problems by prior planning and putting in place appropriate monitoring and treatment protocols when, for example, generating a new genetically modified animal for the first time or carrying out a new procedure. |

<table>
<tr><td>PROJECT 3</td><td colspan="3">The aetiology of metabolic disorders</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Diabetes, obesity, insulin secretion, channelopathy</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 section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</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">Diabetes is reaching epidemic proportions in Western societies: it already affects 350 million people worldwide (>5% of the UK population) and costs the NHS £27 million a day. A key element in the development of the disease is the inability of the beta-cells of the pancreas to secrete enough insulin when the blood sugar concentration rises. Knowledge of how insulin secretion is controlled is therefore of fundamental importance. One aim of this project is to define the molecular mechanisms that control insulin release and what goes wrong with these processes in type 2 diabetes and neonatal diabetes. Because obesity dramatically increases diabetes risk we also aim to determine how genes that predispose to obesity exert their actions.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td colspan="3">This project will provide novel information about the molecular basis of both type 2 diabetes (the most</td></tr>
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| project (how science could be advanced or humans or animals could benefit from the project)? | common form of the disease) and neonatal diabetes (a rare genetic type of diabetes that manifests soon after birth). The results are likely to be of clinical value and we will strive to ensure that, where possible, they will be rapidly translated into clinical practice. These studies are also likely to yield novel information about the regulation of body weight and possibly suggest novel targets for therapeutic regulation of obesity. Given the recent dramatic rise in obesity (~65% of the UK population are overweight) this could be of considerable importance. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Mice, ~30,000 over 5 years<br><br>Rats, ~400 over 5 years<br><br>Xenopus, 60 over 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? | We will study mice carrying a mutation that causes neonatal diabetes and developmental delay in humans. We will turn on the gene in selected tissues, one at a time, to determine the cause of the diabetes and the neurological problems experienced by patients. Animals will be given (i) simple non-invasive tests of locomotor activity, learning and memory; or (ii) tests similar to those used to study blood glucose regulation in humans (e.g. we will inject a small amount of glucose and then take a 3-4 blood samples to measure the change in blood glucose concentration over time). We will also study how diabetes affects the structure and function of the pancreatic islet cells. A few animals may undego recovery surgery, for example to implant a pill that controls their diabetes. In these cases, perioperative analagesia will be used. Similarly, we will study mice carrying mutations thought to be involved in human obesity. Mice that develop diabetes or obesity will be carefully monitored. Thus the expected severity level of these studies will be mild to moderate. All animals will be humanely killed at the end of the experiment. |
| **Application of the 3Rs** | |

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| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | The use of animals is essential to understand the basis of human disease at the systems and whole organism level, and to provide a link between *in vitro* studies and clinical disease. For a multi-organ disease, like diabetes or obesity, there is no substitute for animal studies. It would be neither permissible nor ethical to carry out the necessary procedures in humans, and simulations cannot provide answers to the questions we seek to address. Studies of genetically modified mice are of considerable value in this respect. For example, mice carrying a mutation that causes neonatal diabetes in humans should help us understand precisely what causes the human phenotype. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | Appropriate calculations will be performed in order to obtain statistically relevant results and to ensure that the maximum amount of scientific information is obtained from each individual animal.<br><br>Where animals have only been subjected to minimally disruptive procedures (such as locomotor tests), they will subsequently be used for other procedures. As far as possible, when animals used in procedures are sacrificed, their tissues will be used for cell and isolated tissue studies. This should help keep animal use to a minimum. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | The mouse is the lowest vertebrate with enough aspects of its genetics, anatomy and physiology shared with humans to generate biologically relevant data that ultimately can be extended to our understanding of diabetes/obesity in humans<br><br>The mutant mouse models we will use will be those that are relevant to (i) understanding human diabetes and obesity and that display common phenotypic and pathological features; or (ii) that can be used to address basic biological questions about the normal regulation of whole body metabolism, glucose homeostasis, and body weight. For example, we will use a mouse model that mimics human neonatal diabetes. |

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| | All terminal procedures will be carried out under appropriate levels of anaesthesia. Whenever an animal has surgery it will receive pre- and/or post-operative analgesia as appropriate. |

<table>
<tr><td>PROJECT 4</td><td>Islet pretreatment to reduce hypoxic graft damage</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Diabetes, islets, transplantation, hypoxia</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)</td><td>
<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</td><td>Yes</td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Insulin is the most important hormone to regulate blood sugar in the human body. It is produced by small cell clumps called islets of Langerhans located in the pancreas. If insulin production is insufficient the blood sugar can not be transported into muscles, fat tissue or the brain finally leading to life-threatening diabetic coma. Type 1 diabetic patients are usually treated with insulin injections several times a day bearing the risk to experience episodes of extremely low blood sugar and to fall into coma. If diabetic patients do not have warning symptoms for these life-threatening events, the only way of cure is transplantation of islets. The highest benefit can be expected when diabetics are transplanted with islets in early stages of the disease.<br><br>To prevent rejection of transplanted tissue in the recipient a potent immunosuppression is required frequently associated with severe adverse events</td></tr>
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| | and long term side effects such as cancer.<br><br>Currently, the only way to protect transplanted cells from rejection without using immunosuppression is encapsulation in immunoprotective devices preventing contact between host´s immune cells and donor tissue. Unfortunately, the specification of currently available devices to provide sufficient oxygen to hosted cells does not meet the enormous demand of insulin-producing islet cells for oxygen.<br>The overall aim of this proposal is to improve survival of encapsulated human islets after transplantation in order to restore normal blood sugar in diabetic patients using two major strategies: 1) islet pharmacological pretreatment utilising biochemical, nutritive or medical compounds; 2) stimulation of self-defence mechanisms by exposure of isolated islets to short periods of oxygen deficiency. |
| 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)? | A continuously increasing population is suffering from diabetes-related diseases and early death worldwide. Reduction of late complications in susceptible patients using islet transplantation without immunosuppression will significantly reduce the tremendous costs for the healthcare economies in the U.K. While previous efforts have been exlusively focused on optimising the technical design of immunoprotective devices no studies are available investigating islet pretreatment for improvement of cell survival in those devices.<br><br>In the case that protective compounds or pretreatments can be identified in the intended study we expect a fast implementation in clinical islet transplantation because only compounds that are consumed for human nutrition or that are already in use for clinical purposes either as licenced medical drug, cell culture medium supplement or component of organ preservation solutions will be trialled in our experiments. Since we are collaborating with specialised companies we expect that significant findings will be made available as medical products ready to be used by other groups working on transplantation of diabetic patients. |

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| What species and approximate numbers of animals do you expect to use over what period of time? | 550 Nude mice and 250 rats used within 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? | Adverse effects that can occur are related to the injection of alloxan, an agent needed to make mice diabetic. Except a moderate weight loss caused by increased urination no side effects are expected from this diabetogenic treatment in a short period of time until transplantation as long as the animals have free access to food and water. The likelihood that animals experience ill health before transplantation is approximately 3%. After transplantation, the quality of transplanted islets mainly determines the condition of the mice which can range from perfect health i.e. weight gain and low blood sugar to complete transplant failure. Transplant failure can result in dehydration and weight loss of moderate severity. Therefore, affected animals will be rapidly killed by a Schedule 1 method. The same applies to all transplanted mice at the end of the experiments. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives. | None of the tests being currently in use for extensive islet quality testing is predictive for function of transplanted islets. It is current consent that islet transplantation in immune-deficient mice is the only assay that significantly correlates with clinical outcome in transplanted diabetic patients. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals. | The project will be performed stepwise. First of all, all compounds or treatments eill be tested in a rigid selection process in vitro to filter only the most efficient compounds or pretreatment before being forwarded to transplant experiments.<br><br>The majority of experiments will be performed using human islets. Nevertheless, the inconsistent supply with pancreases from human organ donors requires to perform the initial experiments with rat islets. The rat pancreas yields the four- to five-fold amount of islets compared to mice. Therefore, significantly |

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| | more experiments can be performed using rats instead of mice as donors. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | We will try to identify and obtain a rat strain that can yield twice as many islets as other strains. By this, we seek to reduce the number of donor rats required for experiments.<br><br>Islet will be transplanted into nude mice because these animals have a defective immune system and accept transplanted tissue without immunosuppression. We will select a strain of nude mice we have extensive experiences with and can partially draw on retrospective data from previous experiments.<br><br>Animals will be rendered diabetic by injection of alloxan which causes a much lower proportion of side effects (3%) in comparison to streptozotocin (25%), another drug that is in use for diabetes induction in rodents.<br><br>In order to minimise animal suffering, the present proposal intends to implant islets under the skin of the scruff region of the neck. In contrast to the kidney capsule, representing the standard site for islet transplantation in rodents, the access to this site is less traumatic using simple needle injection and does not require major surgery and treatment for pain. |

<table>
<tr><td>PROJECT 5</td><td>Oral delivery of test agents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Oral gavage, diabetes therapy</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)</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>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</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We are examining the potential for rationally designed molecules to alter the permeability properties of the small intestine for the improved uptake of biopharmaceuticals, such as insulin, that currently must be administered to patients by injection.</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 benefit of these studies would be the identification of a method to manage diabetics using oral dosage forms for drugs, such as insulin, that are currently given by subcutaneous injection. The information obtained from these studies may also provide an improved understanding of how the small intestine functions in health and disease.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Rats and mice will be used. We plan to use less than 390 rats and less than 390 mice over this five year period.</td></tr>
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| over what period of time? | |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Single and repeat dosing studies will be performed by oral gavage. This is a standard protocol and we have extensive previous experience with it. While the animals experience some discomfort, the procedure is over in less than 1 min. Adverse effects are primarily focused on poor placement of the dosing needle used for this procedure, which results in delivery to the lungs instead of the stomach. If this occurs, animals will have laboured breathing and show other indices of stress such as lack of movement. If this occurs, the animal will be euthanized by a Schedule 1 method.<br><br>The agents to be tested will have been shown to be safe and efficacious for the enhanced uptake of biopharmaceuticals across the mucosa of the small intestine. If toxicity of a modulating agent is observed (as demonstrated by stress indices of ruffled fur, decreased movement, increased levels of pro-inflammatory cytokines, etc.), additional rats (no more than 20 in total) will be used to find non-toxic doses of these modulating agents for subsequent testing. We will perform studies with the modulating agent alone as controls to specifically interrogate potential toxicity questions.<br><br>Our protocols will result in blood draws that are below the Home Office allowance; 10% of blood volume on one occasion and no more than 15% blood volume in a 28-day period.<br><br>At the end of the study animals will be euthanized by a Schedule 1 method. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Only whole animals provide the complexity of the small intestinal barrier organized with portal and systemic circulations. |
| **2. Reduction**<br><br>Explain how you will assure | All of the modulating agents to be tested *in vivo* have first gone through extensive *in vitro* studies |

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| the use of minimum numbers of animals | that screens out those that are not effective or are cytotoxic. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Rat is the smallest animal with anatomy and physiology similar to man that can be used for repeated oral gavage and where sufficient amounts of blood can be withdrawn to achieve accurate measurement of the drug that was absorbed from the intestine. Mouse is the smallest animal with anatomy and physiology similar to man that can be used for repeated oral gavage where strains of these animals have been identified that can be used to model certain human conditions and diseases. Measures to optimize animal welfare will include providing water *ad lib* throughout the study, minimized time windows for food restriction, pre-screening of the agents to be tested for toxicology and efficacy. |

| **PROJECT 6** | **Neuroplasticity, immunity and behaviour** | |
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| Key Words (max. 5 words) | rodent, seasonality, leukocytes, reproduction, neuroangiogenesis | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3) | X | Basic research |
| | | 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 |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | The objectives of this Project licence are centred on the role of light and hormones for the regulation of brain plasticity; and how gene expression impacts behaviour and immune function, To achieve this, we take advantage of the naturally occurring changes in gene expression in the rodent brain and immune cells. The specific goals of the studies arc to uncover new genes that may increase (or prevent) brain function and immune responses via angiogenetic mechanisms. | |
| 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)? | The primary benefit of the work is the advancement of scientific knowledge on changes in gene expression in the brain and immune cells ovcr the next 5-10 years. The knowledge gained will be directly relevant for human and animal health and welfare. Humans and animals exhibit seasonal rhythms in mood disorders, suicide, infections, and disease. By discovering new ways in which genes are controlled, the work might help identify new light mediated approaches for human and animal care. | |

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| What species and approximate numbers of animals do you expect to use over what period of time? | This project licence will use 600 hamsters over 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? | Animals may be injected with physiological doses of hormone or pharmaceutical agents. This is a mild procedure that very rarely exhibit adverse effects. In sonic cases, hormone treatments may lead to reduced body weight. Some animals may experience surgery. Pain and suffering is minimised by good surgical and aseptic techniques, suitable anaesthesia, good penoperative care and adequate provision of pain relief. Any animal showing any signs of ill health will he closely monitored, receive veterinary treatment or will be humanely euthanized. In the end, animals will experience Schedule I methods for tissue and data collection. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Animals are the only model for the proposed licence because it is not possible to study the brain using cultured cells or computer modelling. At present, to understand how external factors such as light affect behaviour, the brain, and immune interactions requires the use of live animals. The research programme seeks to identify means to replace gene-behaviour studies by incorporating mathematical modelling when available. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | Sample sizes arc based on statistical power analysis from several prior experiments and power calculations conducted with a statistician. When power values are observed to be consistently high (>0.8); sample sizes will be reduced to include the minimum number of hamsters necessary. Built into the experimental design and dissemination of the results are the ARRIVE guidelines established by the NC3Rs. Measures taken to avoid unjustified duplication of procedures will include close monitoring of literature; conference aaendance and discussing current procedures with colleagues and veterinary staff. |

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| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Hamsters are the best species to study seasonal rhythms in physiology, behaviour and immune function. There is a large scientific literature on this species and alternative models (for example rats and mice) do not exhibit the large brain, physiological and immune changes over seasonal time scales. Refinements for injections include the implementation of scoring sheets and humane endpoints. Pain and suffering is minimised by good surgical and aseptic techniques, suitable anaesthesia, good pen-operative care and adequate provision of pain relief. To prevent duplication of experimentation, scientific conferences are attended and discussion held with colleagues. The scientific literature is continually reviewed and veterinarians consulted for alternative surgical treatments and novel means to alleviate adverse effects. |

<table>
<tr><td>PROJECT 7</td><td colspan="3">Metabolic alterations of pregnancy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Pregnancy, metabolic disease, offspring, therapies</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)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</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">Pregnancy is associated with a series of metabolic changes in the mother that are necessary to support the nutritional needs of the developing baby. These can have consequences for the health of the pregnant woman and her baby during pregnancy and in later life. In normal pregnancy, these changes include raised cholesterol levels as well as increased insulin resistance, a condition that usually leads to diabetes, and high blood levels of bile acids (chemicals made by the liver as a way to remove cholesterol from the body).<br><br>In high-risk women, these changes cause metabolic disease of pregnancy. Metabolic disease of pregnancy can cause increased rates of sickness and death of the pregnant woman and her baby. They also have implications for the subsequent</td></tr>
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| | health of the children of affected pregnancies. Moreover, metabolic changes in pregnancy may have important health consequences for women who do not have diseases of pregnancy *e.g.* women who have had a large number of pregnancies have an increased risk of developing heart disease in later life, and this is thought to be due to continuous exposure to raised levels of cholesterol.<br><br>This work aims to elucidate the factors that drive gestational metabolic changes and how these factors can lead to metabolic disease of pregnancy. The impact on the embryo and children will be also determined. Additional experiments will enable evaluation of therapies that can be applied to prevent metabolic disease in pregnancy. |
| 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)? | The proposed research will have an impact on human health for a wide spectrum of individuals. The results will be of relevance to women with metabolic pregnancy disorders, *e.g*. gestational diabetes, cholestasis and obesity. Children of affected pregnancies who are more susceptible to metabolic syndrome may benefit from this work. There will also be economic benefits to the NHS if this research identifies effective treatments to reduce metabolic disease of pregnancy and susceptibility of children and young adults to metabolic syndrome. This work will inform affected women of ways they can improve the subsequent health of their children. Pharmaceutical companies that invest in strategies to prevent obesity, diabetes and fatty liver will benefit from our proposed research. This research is investigating factors that are involved in the aetiology of these diseases, and will provide insights into strategies that could be tackled by drugs or other therapeutic interventions in young adults that are susceptible to metabolic syndrome. The work will also have an impact in the field of the developmental origins of health and disease, as we have developed new experiments to investigate factors of pregnancy that cause subsequent susceptibility of the children of affected |

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| | pregnancies to metabolic syndrome. |
| What species and approximate numbers of animals do you expect to use over what period of time? | The species we expect to use are mice. The estimate number for the duration of the project is 8000. |
| 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 research plan involves mating of animals and characterisation of the metabolic profile of the offspring through collection of organs after killing the animals in a humane way. In the cases of more invasive methods, such as surgical procedures *e.g.* to remove reproductive organs or supply a compound or imaging, general anaesthetics will be used in combination with anaelgesics, painkillers and proper post-operative care to keep pain and suffering in the absolute minimum. Surgery will be carried out using the same kind of aseptic techniques that are used to avoid infection in human operating theatres. Special diets and other non-invasive methods such as routine tests to assess glucose and insulin function that will be used in this research are not expected to cause any pain and animals will be treated in a humane way in every occasion. No animal is expected to experience more than moderate severity and many will experience no more than mild. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | We will employ non-animal experimental tools as alternatives to the use of live animals wherever possible, For studies of metabolic alterations, we have an active human research programme to collect samples from pregnant women and the fetus where possible from cholestatic cases and non-pregnant controls. This includes blood, urine, faeces, placenta, intestine, liver and uterine biopsies, fetal samples and amniotic fluid. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers | Based on the animal data, we always aim to reflect findings at the clinical level by collection of human samples (*e.g*. blood, urine, faeces and placenta where feasible) or by performing population studies |

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| of animals | or by developing non-animal tools with human resources where appropriate. Moreover, the proposed experimental designs and methods of analysis are always discussed with statisticians so that we can maximise the information obtained from the minimum resource. Also, more than one researchers share the same animals to address their questions. In this way, we aim to minimise the numbers of animals used for our studies. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | It is necessary to use mice with pregnancy disease to investigate the aetiology of metabolic disorders of pregnancy as it is not possible to obtain liver, pancreas and fat from pregnant women and their children. Moreover, use of animals is a useful method to determine causes of disease as genetic and lifestyle influence, often referred to in population studies, can be eliminated. This will allow better evaluation of data and more solid conclusions to be drawn. Also, based on studies performed by the applicant and others, there is already a considerable amount of background information on the hormonal and metabolic parameters of mice that will facilitate experimental planning and validation of the results.<br>Our research plan involves mating of animals and screen of metabolic profile through collection of samples after killing the animals in a humane way. In the cases of more invasive methods general anaesthetics will be used in combination with anaelgesics, painkillers and proper post-operative care to keep pain and suffering to the absolute minimum. Special diets and other non-invasive procedures such as routine glucose and insulin tolerance tests that will be used in this research are not expected to cause any pain. |

| **PROJECT 8** | **Modulation of endocrine pathways by imprinted genes** | |
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| Key Words (max. 5 words) | Imprinting, hormones, development, epigenetics, metabolic. | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3) | 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 |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | Pregnancy is associated with high rates of morbidity and mortality despite broad advances in healthcare over the last *50* years. In addition, as rates of obesity and diabetes increase in the general population, the outcomes of pregnancy have worsened and maternal and fetal health remains a significant public health issue. Underlying this is a lack understanding of how energy is diverted to the fetus from the mother during gestation, and how this goes wrong as a result of the interaction between altered maternal diet and genetic factors. In addition, there are few diagnostic tools available to detect when the energy supply to the fetus is compromised, and consequently paediatric clinicians have a paucity of information upon which to act to improve outcomes for pregnant women and their children.<br><br>We propose to increase our basic knowledge about how the fetus gains resources from the mother during pregnancy. In late gestation, when the fetus is growing very rapidly, the mother must be able to deliver maximal resources. One way in which the | |

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| | mother's body knows to do this is because the placenta releases hormones into the maternal circulation that signal redistribution of maternal nutrients obtained from food. For example, during late gestation the mother becomes less likely to convert excess dietary glucose into stored fat, and instead the glucose is transported across the placenta to become fuel for fetal growth. We work on a novel signalling protein that increases in concentration in maternal blood during pregnancy in mice. Others have found that the concentration of this protein also increases in the mother's blood during human pregnancy. We recently proved (using genetically modified mice that have a mutation the gene encoding this signal) that this protein must come from the fetus or the placenta. We propose to discover if we have discovered a new placental hormone that signals to the mother to increase the energy supply to the fetus.<br><br>Our protein is encoded by a member of a group of genes that are regulated in an unusual way. Each gene in the genome is present in two copies, one inherited from the father and the other from the mother. In most circumstances both of these genes form the template for producing proteins. However, a group of'—lOO genes in the mammalian genome are only expressed from one parental copy, and the other copy is silenced, the imprinted genes. Imprinted genes are known to encode molecules that have crucial flinctions in growth and development, as well as in metabolic processes during adulthood.<br><br>The maternal pituitary gland is known to change its size and hormone output during pregnancy. The novel hormone, and other imprinted products are expressed in the pituitary gland, and are regulated by pregnancy. We would like to understand if imprinted genes mediate maternal between normal pregnancies and those where fetal growth is compromised. |
| 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)? | It is likely that levels of our novel hormone differ between normal pregnancies and those where fetal growth is compromised such as in preeclampsia and intrauterine growth restriction. Our hope is that by understanding its source and function we could in the future use maternal hormone levels as a novel non-invasive marker of fetal well-being, by informing clinical practice to improve pregnancy outcomes. |

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| | More widely, we hope to understand more about the basic science of maternal-fetal communication during pregnancy. This knowledge could lead to additional benefits to understand why pregnancies go wrong, and how to diagnose complications early before maternal and fetal health are compromised. |
| What species and approximate numbers of animals do you expect to use over what period of time? | Mice. We expect to use a maximum of 9000 mice over *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? | Here we will generate and use mice that are defective in genes regulating these important pathways and conduct studies to understand how they work to affect the health and wellbeing of the mice and their offspring. Protocols include metabolic analyses and tests of hormone production, and following the growth and development of the mutant mouse. The majority of these procedures *(>95%)* are expected to cause minimal adverse effects (mild or less). In a minority of cases the animal may experience moderate severity *(<5%),* for example as the result of surgery. In addition, some genetic modifications may cause phenotypes with moderate severity, such as changes in body weight or appetite regulation. These animals will be closely monitored to minimise secondary adverse effects. At the end of the experimental protocols animals will be killed by humane methods. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Because we are studying interactions between mother and fetus during pregnancy we are unable to conduct them in a cell culture system. In addition, hormonal systems often involve the interaction between multiple organs in the body, so the use of an intact animal is essential to understand how they work. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | We have worked with mice for over 15 years and have good experience of colony management. This means we will only breed the minimum number of animals necessary to provide statistical rigour for our work. We will share material between projects, so we do not need to generate multiple sets of experimental |

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| | animals. Where possible we will use cultured cells to test some of our hypotheses before moving into the animal experiments. Also, we can use cells for further experiments to get more details on pathways that are being affected in our mutant mice that cause their abnormalities. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Despite some important differences between humans and rodents, mice have been used for many years to study pregnancy and hormonal regulation. Since the DNA of mice has been sequenced, they are widely used for genetic manipulation, which can now be conducted with great precision. On the rare occasions when surgical techniques are necessary, we will employ analgesic agents as recommended by our NVS. We will minimise the chances of unwanted, severe outcomes from genetic manipulation by testing outcomes at early stages of the process; in cell culture and in very young embryos. |

<table>
<tr><td>PROJECT 9</td><td colspan="3">Relationship between diabetes and cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Diabetes, Cancer, Pancreatitis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3"></td></tr>
<tr><td>Purpose of the project (as in Article 5)</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</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">In this project we propose to study how diabetes and cancer may be linked. Diabetes and obesity are on the rise and whilst we are interested in generating treatment for these diseases, we also need to be wary that both diseases are linked to an increased risk of cancer. In addition some anti-diabetes drugs have been alleged to promote cancer development. Our aim in this project is to study both how dysfunction of the endocrine pancreas- the part of the pancreas which produces the pancreatic hormones, e.g. insulin and glucagon- leads to diabetes, and how this may be linked to cancer development. We plan to study the pathways that lead to endocrine pancreas dysfunction and diabetes, and how these may relate to cancer development. We place emphasis on the study of pancreatic cancer as the prognosis for this type of cancer is particularly poor, and certain anti-diabetic drugs have recently been</td></tr>
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| | shown to lead to increased pancreatitis, a precursor to the development of pancreatic cancer. |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)? | We will study how dysregulation of endocrine pancreas function may lead to diabetes, and how these pathways may relate to cancer development. We will also investigate whether some anti-diabetic drugs may promote cancer development and, if so, how. |
| What species and approximate numbers of animals do you expect to use over what period of time? | We expect to use mice (10000) and rats (5000) over the course of the project licence. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | Our protocols will be of a low to moderate level of severity. In brief we will be inducing diabetes with administration of diets with high caloric value and/or chemical/genetic manipulation. We will then try to reverse diabetes using drug treatment, and/or alteration of diet regiment. Treatment and monitoring of diabetes will require the use of procedures similar to those used in human patients. Animals will be killed by Schedule 1 at the end of the project period. Animals exhibiting any unexpected harmful phenotypes will be killed, or in the case of individual animals of particular scientific interest, advice will be sought from the local Home Office Inspector. Any animal displaying signs of ill health will be examined by the *in house* vet. If the animal fails to respond to treatment or its condition deteriorates, it will be humanely killed by a Schedule 1 method. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | The maintenance of normal blood glucose requires the interplay between hormonal secretion from the islets of Langerhans and hormonal action on peripheral tissues such as the liver, the skeletal muscle and adipose tissues. Neuronal outputs from the brain in response to changes in hormonal signalling and nutrient availability also modify the net effect on blood glucose. Such complex interrelations cannot be reproduced *in vitro* and |

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| | require a whole living organism. Although we do most of our work on cell lines and freshly isolated islets of Langerhans from animals humanely killed by Schedule 1 methods, we ultimately need to validate the effects on hormonal secretion and glucose homeostasis in the whole animal. Validation of the effects of anti-diabetic drugs on the propensity for the development of cancer e.g. risk for pancreatitis and pancreatic cancer, can only be assessed in the whole animal. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | For most of the quantitative experiments, sample sizes will be set using careful statistical analysis. We will use the least number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from other published reports). Usually 6-8 animals per treatment group are sufficient to obtain the required results. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Mice are the lowest vertebrates in which genetic manipulation can be successfully achieved and where diabetes studies are well documented. Rats give a better yield of blood and tissues per animal than mice and are preferred when we do not need to use transgenic animals. All the procedures in this licence are classified as either mild or moderate and are done under local, general or terminal anaesthesia, where appropriate, to minimise stress and suffering of the animals. Where appropriate, pain relief will be provided. |

<table>
<tr><td>PROJECT 10</td><td>Energy Homeostasis and Metabolism</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Food intake, energy expenditure, obesity</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)</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</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Energy homeostasis is the balance between energy intake and energy expenditure. It is regulated by a number of different organs in the body, including the gut, body fat and the brain. This project is designed to elucidate the complex mechanisms by which these different organs interact to regulate energy intake and energy expenditure.<br><br>The objectives of this programme of work are to:<br><br>1) Determine the effects of the short and long term administration of specific signalling molecules, for example, hormone or nutrients, on food intake and energy expenditure in rodents.<br><br>2) Investigate the mechanisms by which such signalling molecules influence food intake, energy expenditure, body weight, activity and metabolism in rodents.<br><br>Understanding the effects of these signalling</td></tr>
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| | molecules and how they work to influence energy homeostasis will help determine their potential as targets for anti-obesity therapies. |
| 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 is a major medical problem in Western society, and results from a long-term disturbance of energy balance, i.e. the energy taken in as food is greater than the energy expended. Currently it is estimated that there are over 1 billion people overweight worldwide. It is estimated that in the UK alone, obesity causes 30,000 premature deaths a year.<br><br>Studying the systems that regulate food intake and energy expenditure will improve our understanding of how they interact to regulate body weight, and how their dysfunction can result in obesity and metabolic diseases such as diabetes. Identifying the most important signals and understanding their effect on the body will aid the development of novel effective therapies for obesity and metabolic disease. |
| What species and approximate numbers of animals do you expect to use over what period of time? | I expect to use 5475 mice and 2950 rats over a five year period. |
| 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? | None of the proposed studies is aimed to result in adverse events, and none should result in events of more than moderate severity. All surgery will be carried out using appropriate anaesthesia and analgesia. Animals showing unacceptable responses to substances administered, treatments given, surgeries performed or genetic changes will be humanely killed. All animals will be humanely killed at the end of the study. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Energy homeostasis involves the interactions of multiple body organs and systems. Its study thus requires the investigation of whole animal physiology. Initially, it is neither ethical nor possible to perform these experiments on humans. There is |

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| | therefore no viable alternative to the use of animals. When possible and appropriate, substances will be initially characterised using cell lines and other non-animal methods. Where tissue level mechanisms are being investigated, it will also be possible to use tissue from animals rather than whole animals. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | All animal experiments will be carefully planned. The natural variation in food intake and body weight between rodents of the same species and genotype can necessitate relatively large group sizes to detect effects. Statistical tests will be carried out to ensure only the minimum number of animals required for each study is used. Where practicable, at the end of the studies the maximum number of tissues will be used from each animal to minimise the numbers required. The maintenance of transgenic breeding colonies, the breeding of different lines together, and the need to investigate the effects of specific genes in one particular sex, can necessitate the breeding of relatively large numbers of transgenic animals. Breeding strategies for genetically altered animals will be optimised to minimise the number utilised. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Mice and rats are the species most widely used to study energy homeostasis and metabolism. The systems that regulate these functions are very similar in rodents and humans, and there is a lot of background knowledge on how they work in rodents, which reduces the number of experiments that need to be carried out. For all studies, anaesthesia and analgesia will be used where appropriate to minimise welfare costs. |

<table>
<tr><td>PROJECT 11</td><td>Effects of mitochondrial functioning on mammal phenotype</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Endothermy, BAT, oxidative stress, rodents</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Understanding how and why particular individuals have greater number of descendent offspring with genes for a particular trait in the following generation (i.e. greater fitness) is at the heart of ecology and evolutionary biology. But one key point is that the offspring that bear those genes are made of flesh and blood. Thus, their production is set by the rate at which parents and their offspring can convert food into energy, the so-called metabolic rate, to invest into reproduction but also growth and survival. Mitochondria are small organelles that are responsible for the conversion of food (substrates as glucose, lipids and proteins) into energy usable by the cells. In other words, mitochondria are the powerhouse of the organism, and energy conversion relies on a “respiratory chain” (i.e. the engine) that is enclosed in the mitochondria. Interestingly, mitochondria have their own genome that encodes for enzymes (i.e. pieces) of the respiratory chain. The proper functioning of the respiratory chain relies nonetheless also on enzymes encoded in the</td></tr>
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| | nuclear genome, and therefore the two genomes (mitochondrial & nuclear) have to cooperate to produce energy. Mitochondrial functioning is also tightly linked to the evolution of endothermy, mitochondria playing also a central in the conversion of food into heat. Endotherms have indeed cells with high densities of mitochondria that are consuming large amount of energy to maintain body temperature constant whatever the ambient temperature. In mammals, the brown adipose tissue (BAT) is the main organ accounting for adaptive heat production. Using small endotherms, this proposal aims at (i) testing nuclear by mitochondrial interaction and climate as proximate sources of variation in mitochondrial and whole organism metabolic rate, and (ii) exploring the life history consequences. |
| 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)? | Effects of alteration to food macro nutrients and climate on the variation of behaviours and life histories of endotherms has been little investigated and virtually nothing is known about the relative contribution of the mitochondrial genome and the nuclear genome in shaping the behavioural and life history adaption of endotherms to food and climate changes. This project should provide novel insights on the role of the mitochondria on the organismal phenotype of endotherms. It should also yield vital information for predicting the distribution of endotherms and their response to climate change. Furthermore, the 're-discovery' of BAT in adult humans, and its possible health effects on obesity, diabetes or cancer has led to enormous interest on this tissue. This project should provide novel insights on the 'ecological significance' of BAT in endotherms as well as the 'correlated evolutionary consequences' of BAT activation on animal health and behaviours. |
| What species and approximate numbers of animals do you expect to use over what period of time? | This project licence will use *ca*. 2100 voles over 5 years, individuals being usually involved in research protocols for a period of ca. 6 months. |
| In the context of what you propose to do to the animals, what are the | This licence contains studies that may include selective breeding on traits of interests and where individuals may be subject to changes in housing |

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| expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | temperatures and/or diets and/or photoperiods, and affects may be measured through metabolic analyses, behavioural analyses and blood parameters As a result, animals may show alteration of their metabolism, behaviour and body. Animals will be humanely euthanized for tissue harvesting and analysis at the end of this licence. |
| **Application of the 3Rs** | |
| **1. Replacement**<br>State why you need to use animals and why you cannot use non-animal alternatives | Those questions can only be addressed using live endotherms. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | Experiments are designed on the basis of our own findings or published data. Prior to any new study a full evaluation of previous/pilot data will be carried out and we will also perform calculations to work out the minimum number of animals required to obtain valid data. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Rodents are the most suitable endotherm species to perform selective breeding experiments.<br>This project aims to minimise all forms of distress and discomfort in the rodents by making use of the wealth of literature on good practices in rodent research and of our previous experience working with voles. In the unlikely event that animal welfare is under consideration, all available resources will be consulted and recommendations will be immediately implemented. |

| **PROJECT 12** | **Nuclear envelope roles in health and disease** | |
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| Key Words (max. 5 words) | nuclear envelope, genorne organ isation, metabolism 5 years | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3) | 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 |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | The organisation of the DNA in the cell nucleus has different patterns for each type of tissue in the body. We have evidence that much of this patterning is directed by nuclear envelope, a structure of protein and membrane that separates the nucleus from the rest of the cell. The goal of this project is to understand the role of nuclear envelope proteins in establishing these organisational patterns and their impact on gene expression during development of the organism.<br><br>Preliminary data from our lab and others suggest that tissue specific patterns of genome organization contribute to normal tissue development and tissue specific cell functions. This work should help us to understand several human diseases caused by mutations in nuclear envelope proteins e.g. diabetes, obesity and lipodystrophy. | |
| What are the potential benefits likely to derive from this project (how science could be advanced or humans or | Nuclear envelope proteins have been linked to a wide range of human diseases including lipodystrophy, muscular dystrophies, blood disorders, neuropathies and complex diseases such as premature ageing. Moreover, our main focus, the *TMEM12OA* gene has | |

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| animals could benefit from the project)? | been linked to obesity and diabetes. Therefore, there is potential to translate our results into therapies for these major health concerns. Tmeml2OA may also mediate nuclear envelope-linked lipodystrophy. Lipodystrophies are diseases characterized by local or general loss of fat tissue, associated with metabolic syndromes including insulin-resistant diabetes, dyslipidaemia, and nonalcoholic fatty liver disease. Nuclear envelope-linked lipodystrophies are caused by mutations in lamin A, a nuclear envelope protein; however lam in A is expressed in nearly all tissues, so it is unclear how the fat-specific pathology develops. Our finding that tissue specific nuclear envelope protein Tmeml2OA binds to lamin A and that Tmeml2OA fine-tunes' gene expression in fat cells makes it a reasonable candidate for mediating nuclear envelope-linked lipodystrophy so better understanding its function could lead to therapies also for this disease. |
| What species and approximate numbers of animals do you expect to use over what period of time? | To achieve our research goal we will use genetically altered mice on a C57BL/6 background. We use wild type animals of this strain as well as genetically altered mice carrying a knock-out or overexpression of nuclear envelope protein genes. In total over the 5 years we will use less than 4000 animals under this 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? | The majority of animals will be used for breeding only, and no major adverse effects are expected in association with the genetic alterations. Only limited number of mice will undergo surgical procedures (vasectomy, embryo transfer) but adverse effects will be avoided by using skilled personnel and appropriate anaesthesia and analgesia. We critically assessed all procedures performed under this licence and severity limits will range from mild to moderate. There is no protocol involving prolonged and severe pain for animals. Each procedure will be followed by frequent welfare assessment and animals showing adverse effects will be humanely euthanized. Moderate severity level will be potentially reached only during generation of genetically modified organisms or glucose tolerance and insulin tolerance tests needed to measure the metabolic cost of the loss or overexpression of these genes. However, this severity is only at the level of injections and taking blood. All animals at the end of protocols will be humanely euthanized by Schedule 1 methods and |

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| | tissues will be collected for further analysis. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Over the past five years we have extensively researched these proteins using *in vitro* tissue differentiation systems, but these cannot test metabolism that depends on a complex interplay of factors in a whole organism. The recent link of Tmeml2OA to obesity and diabetes makes it important to now engage studies in animals. That this protein is expressed in brain in addition to fat requires comparison of its roles in both organs. Furthermore to test if Tmeml2OA mediates the tissue-specific pathology of lamin A-linked lipodystrophy requires mating to an existing mouse model for this disease. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | The minimal number of animals needed for experiments has been calculated using estimated statistical parameters and compared with similar types of experiments published previously by our collaborator and others. Additionally, we have combined minimally invasive and non-regulated procedures into the same protocols such as giving high and low fat diets, weighing mice, and performing glucose and insulin tolerance tests so as to minimize the number of animals used. Furthermore individual blood chemistries will be additionally used to measure leptin and adiponectin levels to minimize both sampling and animal use. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Mouse is a well-recognised model for research on obesity and related conditions and several modified C57BL/6 strains exist for mating in our studies, especially the lamin A lipodystrophy model. Mice are also similar to human with regards to fat metabolism. Finally, small rodents show common features of nuclear envelope composition with humans.<br>To minimize pain and stress to the animals, the majority of the experiments will be performed on tissues from humanely euthanized mice. All procedures on living animals will be performed by experienced research staff. If any mice show symptoms of severe pain or distress at any stage of an experiment, the procedure will be terminated and animal euthanized by a Schedule 1 method or referred to the NVS for advice. |

<table>
<tr><td>PROJECT 13</td><td colspan="3">The role of copper in diabetes and ageing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">arteries, kidney, heart, eyes, biomarkers, treatment</td></tr>
<tr><td>Expected duration of the project (years)</td><td colspan="3">Five</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The overall aim of this programme of work is to understand the mechanisms underlying the key functional changes that take place in various organs in diabetes and to develop therapeutic strategies to reverse or prevent these changes. A comparative study using arteries will also be undertaken to investigate any similarities between diabetes and the normal ageing process.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">Diabetes currently affects more than 200 million people worldwide and by 2025 the World Health Organisation estimates 350 million people will have the disease. Approximately one in five diabetic patients will present with some degree of organ complication that will be debilitating and will impact on the patient’s quality of life. These complications can affect the kidney and heart for example, and ultimately lead to organ failure. There is currently no effective treatment for diabetic complications</td></tr>
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| | once established.<br><br>These studies will result in a greater understanding of the mechanisms underlying diabetic organ damage with the realistic prospect of developing novel therapies involving patients in clinical trials. These results will also be of relevance to veterinary practice for those mammals that suffer from diabetes (*e.g.* the domestic cat).<br><br>Cardiovascular diseases (affecting the heart and blood vessels) are the leading cause of mortality and illness in the developed world, being responsible for >40% of all deaths. Hypertension (high blood pressure), which is due largely to abnormalities in blood vessels, is evident in around 30% of adults and is a major risk factor for cardiovascular disorders. There is a need to improve understanding of the underlying pathological changes associated with the development and progression of hypertension. This project will increase the understanding of these processes and will thus aid in the identification of new therapeutic targets for the treatment of cardiovascular diseases.<br><br>This research will be of benefit to academics, policy makers and regulatory bodies with interest in diabetes and cardiovascular disease, public health and disease prevention and the epidemiology of chronic non-communicable diseases.. |
| What species and approximate numbers of animals do you expect to use over what period of time? | 750 rats over a 5-year period. |
| 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? | Rodent models of Type 1 diabetes will be used. The severity is moderate. Animals are monitored regularly. Animals have free access to drinking water and food. Both of these will be provided in extra amounts. Three or more signs of persistent diabetes will be addressed by humane killing of the animal consistent with the law. Animals have raised blood glucose at levels that might be seen in poorly controlled clinical diabetes. Progression of organ |

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| | disease over the time course of the study will be assessed by collection of body fluids that should not cause any tissue damage or undue stress to the animal. Animals will also undergo MRI and PET imaging to look for changes in the size of their organs.<br><br>Animals are euthanized at the end of the protocol and tissues/ body fluids collected for molecular and biochemical analysis. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | Since diabetes is a whole body disease, affecting the hormonal, cardiovascular and nervous systems which all play a role in progression of the disease, it is impossible to wholly mimic diabetes in a culture dish. The complications of diabetes are progressive and take time to develop and are the product of several consequences of poorly controlled diabetes. Hence there is a need for animal models of diabetes. The similarities between the development of diabetes in humans, mice and rats justifies the use of animal models of diabetes to elucidate the mechanisms of the disease process, in characterizing changes in organ systems and assessing the efficacy of therapeutic agents. If any relevant non-animal alternatives become available during the course of the project, we will incorporate these in our studies. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | Experiments are designed on the basis of previous work and published data. Full evaluation of previous/pilot data and statistical calculations will be performed, such that the minimum number of animals required to provide valid data are used (typically 10 per group for therapeutic studies). |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs | The animal of choice for this work is a mammal, the rat. The streptozotocin-induced Type I model of diabetes will be used. The model's advantages and limitations are well known as well as its specific requirements for animal welfare. The results will be integrated into the continuously expanding body of research conducted on this species and model, and contribute to the emergence of a comprehensive understanding of the development of diabetic organ |

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| (harms) to the animals. | damage.<br><br>Numerous strategies are in place to minimize animal suffering. Appropriate anaesthetics and analgesia for any imaging will be used and animals will be handled and acclimatized to minimize any undue stress. |

<table>
<tr><td>PROJECT 14</td><td>The Role of NPPC in Pituitary Development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>C-type natriuretic peptide (CNP)<br>Genetic modification<br>Tissue specific<br>Mouse</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)</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</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The primary aim of the project is to determine the role of C-type natriuretic peptide (CNP) in pituitary development and reproductive function.</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>A better understanding of how CNP affects growth and reproduction may enable the development of better treatments for diseases affecting these systems in both companion animals and man.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>All work will be done in cell culture or in mice. Many of the assays in mice will be carried out under terminal anaesthesia or post-mortem. We expect to use less than 2,000 mice over the 5 project period.</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 genetic modification that removes the action of CNP is likely to lead to growth and reproductive abnormalities. While these differences are unlikely to generate significant suffering it is possible that reduced growth of some parts of the body may lead to problems of moderate severity. Animals will be killed at the end of the studies which in most cases will consist of observations and basic measurements of weight, length etc and then more detailed post-mortem analyses.</td></tr>
<tr><td>Application of the 3Rs</td><td></td></tr>
<tr><td>1. Replacement<br>State why you need to use</td><td>The experimental approaches build on our previous in vitro studies performed with established</td></tr>
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| animals and why you cannot use non-animal alternatives | immortalised cell lines, primary cell lines and finally zebrafish. Our mouse studies represent the final steps in confirming the roles for CNP in anterior pituitary development and function in mammals, and the broader roles for CNP within the CNS. |
| **2. Reduction**<br>Explain how you will assure the use of minimum numbers of animals | After generation and characterization of the novel transgenics by cross-breeding with established transgenic lines with no harmful phenotype, we will freeze any lines for which we have no immediate further use to prevent breeding unnecessary mice. We have a statistician on faculty, who advises us as to power calculations for each individual experimental approach, allowing us to tailor of breeding programmes accordingly. |
| **3. Refinement**<br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | All experiments will be conducted in mice as this species has been the most widely used for genetic manipulation and has the greatest number of spontaneous and induced mutants and genetically modified strains. Such genetically modified animals are essential for understanding gene function.<br><br>By using mice where the genetic modification is limited to a specific tissue will reduce the severity of effects compared to making the genetic modification in all tissues.<br><br>Determination of genetic status will use the least invasive method possible. Husbandry will be modified to reduce the welfare impact of any abnormalities resulting from the genetic manipulation. |

| **PROJECT 15** | **Genetic Toxicology – Chemicals** | |
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| Key Words (max. 5 words) | Mutagen, Genetic Toxicology | |
| Expected duration of the project (yrs) | 5 Years | |
| Purpose of the project as in ASPA section 5C(3) | | Basic research |
| | | Translational and applied research |
| | X | Regulatory use and routine production |
| | X | 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 |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | The objective is to investigate if positive *in vitro* test responses are confirmed the relevant *in vivo* test method. This will allow sponsors identify if test substance is to be labelled as a mutagen. In some instance the in vivo studies are required when there is a risk of possible high human exposure, substance use in food contact materials for instance. | |
| 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)? | The general consensus is that most mutagens have the potential to be carcinogens. Therefore the potential benefits of the project are the identification of *in vivo* mutagens which may induce cancer. | |
| What species and approximate numbers of animals do you expect to use over what period of time? | Rats and mice are recommended species in all the OECD test methods we use.<br><br>During life span of the project licence we expect to use 9000 mice and 3000 rats. | |
| In the context of what you propose to do to the animals, | Expected adverse effects are those associated with the method of administration and the required level of | |

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| what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | handling this will require, and any toxic response to the test substance exhibited by the animal. Care will be taken to ensure that the latter is kept within the severity limits set for the main test protocols. The aim is not to exceed a moderate severity limit. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | At present there are no validated non-animal alternatives available that can satisfy the regulatory authorities for this area of hazard identification. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | Initially by ensuring there is a valid reason to perform the study.<br><br>Reviewing all available toxicity data to ensure the initial dose levels used in the rang-finding test are not excessive.<br><br>Performing all main tests using a single sex if there is no marked difference in toxicity, and getting a valid scientific justification form the Sponsor if they want both sexes to be used.<br><br>If the sponsor is looking at performing two in vivo studies look at performing them in a combined experiment.<br><br>If possible bolting on a genetic toxicology end-point onto a repeat dose toxicity study. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | Both rats and mice are recommended species in the relevant OECD test guidelines. There are many years of research publication demonstrating the suitability of these species in these test systems.<br><br>By careful analysis of all toxicity data available on all test substances the initial dose levels used in the range-finding tests will be set to cause as little harm as possible. Careful monitoring post dosing will also ensure any excessive welfare issues adverse effects are terminated quickly. |

| **PROJECT 16** | **Impact of gut microbiota on type II diabetes** | |
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| Key Words (max. 5 words) | Microbiota, Metabolism, Type II diabetes, Obesity | |
| Expected duration of the project (yrs) | 5 years | |
| Purpose of the project as in ASPA section 5C(3) | 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 |
| | X | Maintenance of colonies of genetically altered animals |
| Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) | It is now admitted that the gut microbiota plays an important role on the host energy metabolism, particularly in the context of the metabolic syndrome. In order to develop optimal personalised nutrition for energy intake, it is proposed to study the role of some specific gut bacteria on the host energy metabolism, particularly on the liver, as it is a crucial organ in glucose and lipid metabolism. These bacteria are also suspected to influence the foetal programming of metabolic diseases such as type II diabetes. This programme of work will therefore also focus on the inheritance of a 'diabetic" microbiota from the mother to its offspring and determine its impact on future health in a rodent model susceptible to developing type II diabetes. | |
| 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)? | On one hand, the development of obesity leading to metabolic syndrome is strongly associated with cardiovascular diseases in Western countries, which results in a growing number of premature deaths. On the other hand, preterm babies or malnourished populations in developing countries must optimise their energy intake. Understanding the molecular links between gut microbiota and the host metabolism will | |

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| | enable the development of personal ised nutrition plans that also consider the right gut microbial balance for a relatively energy efficient ecosystem adapted in a medical context. |
| What species and approximate numbers of animals do you expect to use over what period of time? | We will use exclusively mouse models of type II diabetes and wild type controls We estimate that we will need a maximum of 750 animals over the entire duration of the project. |
| In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end? | All the procedures described in this project licence are expected to be of mild to moderate severity. This includes blood collection and oral gavage that may induce transient pain and discomfort. Some discomfort is also expected as a result of weight gain. All animals will be killed at the end of the experiment. Only some of the pups that have been rejected by the foster mother and assessed to be in good health will be reused as breeders. |
| **Application of the 3Rs** | |
| **1. Replacement**<br><br>State why you need to use animals and why you cannot use non-animal alternatives | This programme of work investigates the relationship between the host and its gut microbiota in the context of type II diabetes. This requires a living organism with a physiology relevant to humans in which type II diabetes can be simulated. As mice have been extensively used over the last 50 years to investigate host-gut microbial relationships, they are considered suitable models for the purpose of this work. Two mouse models will be used: a diet-induced obesity mouse model and a genetic model of type II diabetes. |
| **2. Reduction**<br><br>Explain how you will assure the use of minimum numbers of animals | The primary outcome of this study is to study the impact of bacteria on hepatic metabolism assessed using a metabolic profiling approach. Previous animal experiments have suggested that 7 animals per group were sufficient to ensure statistically meaningful results in this model using similar techniques. A professional statistician will be consulted where appropriate. |
| **3. Refinement**<br><br>Explain the choice of species and why the animal model(s) you will use are the most | As explained above, this programme of work requires the use of a living organism relevant to type II diabetes development in humans. Diet-induced obesity and genetically obese mice are therefore the most appropriate and most refined animal models. |

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| refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals. | We are committed to keep animal discomfort to a minimum and have refined our protocol to limit the number of invasive procedures to a minimum. Although it is necessary to control the dose of live bacteria given to each animal using oral gavage, we will ensure that this procedure is performed by an experienced technician to minimise the risk of adverse effects. Brief inhalation anaesthesia may be used to reduce stress and discomfort as recommended if necessary. It is also planed to collect urine and fecal samples weekly. This will require a brief manipulation of the animals followed by a short isolation time in an individual cage. This will avoid the use of more restrictive and stressful individual metabolic cages. |