Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 26

Projects with a primary purpose of: Basic Research – Endocrine System/Metabolism

Project Titles and keywords

1. **Mouse models of calcium and bone disorders**
    - Calcium homeostasis; bone dysplasias; parathyroid; kidney

2. **Breeding and maintenance of genetically modified and mutant mice**
    - Mice, ‘genetically modified’, Breeding, mutant

3. **Discovery of healthy lean gene mechanisms**
    - Genes, healthy leanness, obesity, diabetes

4. **Genetic control of endocrine development and tumorigenesis**
    - Pituitary, hormones, birth defects, tumours, genetics

5. **Metabolism, Pharmacokinetics and Biomarkers**
    - Metabolism, Pharmacokinetics, Biomarkers, DMPK

6. **The origin of new β-cells in pregnancy**
    - β-cells, pregnancy, transdifferentiation. 4OHT, transgenic

7. **Cancer Pathways in Metabolic Disease**
    - Metabolism, cancer, signalling pathways, therapy

8. **The physiological role of B3 vitamins in energy metabolism**
    - Energy, Vitamin B31 Nicotinamide adenine dinucleotide, mitochondria

9. **Circadian control of behaviour and metabolism**
    - Obesity, diabetes, clock, biological rhythms, physiology

10. **Embryogenesis, stem cells and cell fate decisions**
    - Stem cells, cell fate, embryogenesis

11. **Extracellular regulation of insulin resistance**
    - Insulin; Obesity; Type 2 Diabetes

12. **The mechanisms of ageing and age-related disease**
    - Ageing, age-related disease, healthspan

13. **Endocrine action and endocrine cancer therapy**
    - Androgens, prostate, cancer, endocrine

Project 1	**Mouse models of calcium and bone disorders**	
Key Words (max. 5 words)	Calcium homeostasis; bone dysplasias; parathyroid; kidney.	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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 level of calcium is tightly regulated due to its roles in multiple biological processes, and disturbances of this cause many common diseases, including kidney stones, osteoporosis, cataracts and diabetes. Calcium disturbances may have many underlying causes including defects in the calcium regulatory processes, kidney failure, and tumours, and many of these are not well treated by current drugs. Development of new drugs is hampered by our lack of knowledge of the underlying biology of these diseases, and we aim to understand this in more detail to aid the development of new and improved drugs.	
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)?	Our studies will help to understand genetic causes, cellular pathways and the interactions between different organs, in diseases underlying disturbances of calcium. We will also be able to assess existing and new drugs and dietary alterations, and will provide new model systems in which novel therapies can be tested prior to their use in man.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mouse models, as mice provide rapid and efficient breeding whilst maintaining a high enough degree of similarity to humans. We expect to use approximately 65,000 mice over the five years duration of the licence.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The maximum severity of this licence is expected to be moderate. The adverse effects experienced by any mouse will be monitored closely. The adverse effects of high calcium in the blood include increased drinking and urination, abdominal pain and muscle weakness, and low calcium in the blood are muscle cramps and seizures (rare). Some of the mice may develop skeletal abnormalities, which may reduce their ability to walk normally. Some of the mice may develop tumours, which normally do not cause noticeable symptoms, but may occasionally interfere with normal movement. In order to study these human diseases, we have to allow them to develop in the mice; however, we will take steps wherever possible to reduce the adverse effects. All of the mice will be humanely culled at the end of the procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Regulation of calcium levels requires the interaction of the parathyroid glands, bone and kidneys, via circulating hormones and vitamin D. In addition, calcium has different effects on different organs such as the pancreas. As this represents a whole body system, it is not possible to investigate and understand disease development in isolated cells rather than in whole live animals. In addition, drugs need to be tested in whole animals so that the responses of all the different organs can be studied. However, we are striving to develop cell models that may be able to replace some mice in some preliminary studies. For example, we are trying to immortalise and grow cells taken from mouse organs in the laboratory, and these could be used to test whether new drugs might be effective, before they are tested in live mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all our mouse work, we use statistical analysis to ensure that the minimum number of mice are bred for the study, and that we use only the number of mice that are required to produce meaningful and useful results in order to answer the experimental questions. We are able to study the effects of drugs in multiple organs within an individual animal, for example in mice which develop tumours in more than one organ. Similarly, we can image the same mouse several times to study the development of organs or tumours, rather than using several mice once. We are also trying to establish cells from mice that will grow long-term in the laboratory. These could be used to

	replace some mice for the early stages of testing of new drugs.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Only mice will be used in our studies, and have been chosen as they represent the lowest mammalian species which allow the necessary genetic manipulations and display sufficient similarity to human organs and physiology. We have developed expertise and experience in mouse welfare, and have refined our tests to ensure that the highest quality data is generated for the least welfare cost. We are keen to minimise severity and increase the welfare of these animals. To ensure this, we will use non-invasive tests that only cause temporary discomfort where possible. For administration of drugs, a small pilot study will be undertaken for new drugs, with increased cage observations and welfare checks to ensure that the drug is safe. We also aim to use long acting drugs where possible to reduce the frequency of dosing. During every test, mice are closely observed and anaesthetics or analgesics used when appropriate.

Project 2	**Breeding and maintenance of genetically modified and mutant mice**	
Key Words (max. 5 words)	Mice, ‘genetically modified’, Breeding, mutant	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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)	Genetically modified mice are commonly used for research or as animal models of human diseases. The two most common types are the knockout mouse, where the activity of one, or more, genes are removed and the transgenic mouse, generated to carry addition genetic information. The objective of the licence is to produce and maintain colonies of mice with specific genetic modifications or mutations to: a) advance the knowledge and understanding of metabolic disease including obesity; b) determine the traits a change in a known gene, or genes, may cause; c) supply other projects allowed to use genetically modified animals of this type.	
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)?	Producing genetically modified animals for research allows scientists to understand the mechanisms by which a disease may arise, how to treat the disease or related symptoms and how current or future drugs may work to treat that condition. Studying the difference between genetically modified mice and conventional mice will allow scientists to know what characteristics or disease states that	

	particular change in genetic information produces in an animal.
What species and approximate numbers of animals do you expect to use over what period of time?	It is expected that up to 5000 mice maybe used over the 5 year term of the licence.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	While we do not expect many adverse effects a few mild severity level ones may occur; these include fighting between mating animals, complications of birth or the failure of a mother to feed her offspring and pain associated with obtaining a blood sample. • At the end of the study the animal may be maintained for breeding; • killed in a humane way by a method approved by the Home Office*;* • supplied to other Projects allowed to use animals with the specific genetically modification.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Metabolic diseases, such as diabetes, and their treatment involve multiple organs including brain, muscle and fat, all interacting and all are affected by external factors such as diet quantity and composition, exercise and temperature, as yet only a whole animal can integrate all of these factors.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Only animals of optimal breeding age will be mated, ensuring the minimum number to animals required to provide the offspring required for experimentation or colony maintenance. Previously obtained records on successful pregnancies and litters (from external sources as well as our own records) will help in identifying these numbers.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the lowest recognised animal model suitable for systemic metabolic research applicable to human disorders such as diabetes. The mouse strains derived from the C57Bl/6 strain are generally the most reliable when investigating genetic and environmental causes of metabolic disease. Regular health monitoring will be performed during pregnancy, labour and suckling to ensure that animal welfare issues to not progress to harmful consequences. Specially designed foraging diets and fertility enhancers may be employed to improve fecundity and cage enrichment.

<table>
<tr><td>Project 3</td><td colspan="3">Discovery of healthy lean gene mechanisms</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Genes, healthy leanness, obesity, diabetes</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>
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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 colspan="3">Our objectives are:
1. To determine exactly how certain genes keep us healthy and lean, even when exposed to too many calories .

2. To determine how these healthy lean genes might be targeted with new medicines to treat obesity.

The key objectives are to compare the effects of lean genes with other known causes of healthy leanness (controlled fasting, exposure to the cold, exercise) or to contrast this with causes of unhealthy leanness (lipodystrophy) or obesity.

Finally, we wish to understand how
lean genes might further protect us from the cell damage found with increasing age.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td colspan="3">This research will develop our understanding of how genes lead to healthy leanness. This research may identify new genes and medicines that promote healthy leanness through counteracting the negative effects of obesity and ageing .</td></tr>
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project)?	One new gene identified by this research is already being tested with a targeted medicine in human diabetic patients. Development of animal models with altered lean gene levels in this project will help us understand how these medicines work and help improve how effective they are in living animals.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use various strains of commonly studied laboratory mouse because they share substantial common biology with humans in health and disease. We expect to use 1600-1800 mice over the 5 year period, many of which will be generated by our genetically-altered mouse breeding programmes.
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 the impact of lean genes on obesity-causing and ageing processes that impact metabolic health. To do this we will use diets and genetically altered mouse models that make the mice prone to obesity, or diabetes, or the effects of ageing. We carefully monitor the mice in these studies to prevent exceeding moderate severity levels. Indeed we need to understand how lean genes affect metabolism before major symptoms occur.. In ageing we study the mice at defined middle-aged and old-aged points before 'natural death' through ageing becomes common for that strain. Mice are humanely euthanized once we have gathered the necessary metabolic information
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Metabolism is a highly integrated physiological processes that reflects complex interactions between the brain (e.g. appetite) the adipose tissue (storage of excess fat), muscle (calorie usage), liver (calorie storage and integration), etc. Because of this there is no way to replace the insight that investigating lean gene effects in whole animals provides. Ultimately we must understand what such manipulations would do in living humans. However, we use clonal cell models of several tissue types to test key hypotheses before animal experimentation is considered. For example, cultured fat cells have been used in our research to show that one 'lean gene' prevents defects in release of healthy fat cell hormones. Similar approaches are being taken with cultured human

	liver cells and cultured mouse muscle cells. Whilst this reductionist approach helps us understand how each tissue might contribute to an overall effect, only whole animal studies can show how these changes affect an animals overall health and, importantly, how effective any new drug would be.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use statistics (power calculations, factorial designs) based on extensive experience of our metabolic studies (for e.g. body weight, blood nutrients and hormones) to determine the minimum number of mice needed to confidently measure meaningful differences caused by lean gene alterations or therapy. We routinely use mice that are used commonly in our research community and that are genetically identical to minimise variation in experiments. We will use new non-invasive technologies to determine fat mass and calorie burning capacity of mice that will allow reduction of animal numbers because we can do 'before and after' measurements (pairing of data across longitudinal studies) rather than using two groups of animals. We work with other scientists with projects that allow them to use some of our post-mortem animal tissues to inform on their research on heart, blood vessels, inflammation, reducing the need to use more animals in some cases. Regular meetings of our research group ensures maximal use of our materials and animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use animal models best suited to address the biological question/ under study. For example, mutant obese mice are used for obesity studies. Obesity-prone 'normal' strains of mice are chosen for dietary obesity studies. Mice mutations affecting cholesterol/lipid metabolism are used in combination with high cholesterol diets that focus research questions related to atheroma as a consequence of obesity. These models are used extensively, globally, and are recognized as the mildest interventions possible whilst delivering better cross-centre replication of outcomes (e.g. obesity, atherosclerosis) For surgical procedures, appropriate anaesthetic and pain-killers, and sterile techniques will be used. Drugs will be administered at non-toxic dosages and if unknown, this will be tested in a carefully

	graded dose-finding protocol. The introduction of new non-invasive, low stress procedures for body fat mass determination allows us to minimise suffering while maximising the amount of information obtained from each animal We will use new home cage chambers that allow us to follow metabolism in real-time without interfering with the animal (Indirect calorimetry measures oxygen used/CO2 respired). This removes the need for metabolic cages that have grid features in most studies. We follow a path of progressive method development and refinement. For example, for nutrient metabolism exploratory methods such as oral administration of glucose with blood sampling are used to test for major effects of gene alteration on broad outcomes such as 'does diabetes improve'. Only then if an effects is clear are in depth methods used, such as using infusions of labelled nutrients (e.g. glucose) and tracking what happens to them in a living animal are employed to work out mechanisms of health change. The use of any invasive (e.g. surgical) techniques are discussed with colleagues performing similar work locally and across the country. Monitoring systems will be tailored to each model and strict humane endpoints will be applied to minimise suffering. Training and good practice is encouraged through group meetings and regular discussions with the NVS and key staff.

Project 4	**Genetic control of endocrine development and tumorigenesis**	
Key Words (max. 5 words)	Pituitary, hormones, birth defects, tumours, genetics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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)	This project uses normal and genetically alterea mice to understand the normal development of the brain and pituitary gland as well as the disease associated with these organs. This is important to look at the mechanisms that control normal development and to learn how defective gene function leads to human disease. In particular, this project aims to understand conditions affecting children and that are present at birth (these are termed congenital malformations). The brain, eyes and pituitary gland develop from the front (anterior) regions of the embryo and their normal development is intimately associated. Changes in the genetic constitution (genes) affecting the development of these tissues tend to show abnormalities that impair the functioning of vital organs involved in reproduction, growth or how the body utilises energy from food. For example, congenital hypopituitarism (lack of pituitary function) in humans, which include conditions affecting the activity of one or more pituitary hormones, is usually linked to forebrain and eye defects. Pituitary tumours in children can also be considered as developmental disorders, in which these tumours grow in brain and pituitary gland that during early gestation and early years of life. The growth these tumours disrupts the pituitary and brain from their normal functioning.	

	Hypopituitarism and hypothalamic-pituitary tumours have a significant prevalence in humans and are associated with severe symptoms that negatively affect the quality of life of the patients and often can lead to death. The aim of the proposal is to understand these conditions with the primary goal of improving the patient management and care.
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)?	Congenital malformation of the brain and pituitary an important conditions in humans. The proposed research wil help understand the causes and development of th disease. This will lead to the development of nove diagnostic tools and improved treatment. The direct beneficiaries to the project are the patients that suffer from these conditions and do not have either accurate diagnosis nor proper treatment.
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 7800 mice over 5 years to be able to perform the studies all required numbers for the propose study.
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 the development of the brain and pituitary which are responsible to control multiple physiological functions, such as metabolism, growth, puberty and formation of tumours. Understanding how this process is regulated will help to understand the disease. Protocols include anatomical studies of these structures, tests of hormone production, studies and following the growth and development in the absence of important genes to fully understand its requirement. The majority of these procedures are expected to cause minimal adverse effects (moderate or less). In addition we will use murine models with alterations in their DNA that lead to moderate or mild effects such as changes in body weight or appetite regulation. Surgery will be used in some instances, to challenge or modify the physiology of the body with the final goal to understand how these changes affect the endocrine system. This is important to understand how these changes are regulated with we want to identify novel To be able to understand the effect of lack of these organs in the general endocrine physiology. These animals will be closely monitored to minimise secondary adverse effects.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	•Direct studies of humans tissues can be done at the descriptive level but to understand the development of the organs cannot be done in humans on practical and ethical grounds. Where ever possible we will use • Tissue cell culture: When ever possible we will use tissue culture for cellular studies. •Computer simulations: We will use computer simulation particularly in identification of protein-protein interactions and pathways that are implicated in gene function.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In vitro cell culture: In order to minimise the use of chemical compounds for therapeutic effect, we will use in vitro (cells grown on a dish) before testing on a whole animal system. Careful experimental design: we will use statically analyses and calculation determines sample size, and we will seek professional statistical advice where needed.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mouse genetics is understood almost as well as in humans, offering the best possible means research Birth defects in genetically-predisposed mice closely resemble those in humans, providing excellent models for analysis. Transgenic/gene knockout technologies offer a sophisticated route towards studying the effects of genes in particular tissues, or at specific stages

Project 5	**Metabolism, Pharmacokinetics and Biomarkers**	
Key Words (max. 5 words)	Metabolism, Pharmacokinetics, Biomarkers, DMPK	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the work carried out under this Project Licence is to support the discovery of medicines for unmet medical needs, Specifically, experiments will be conducted to determine and understand what the body does to novel therapeutic agents in laboratory animals and their concentrations within tissues of interest. This information is necessary to select optimal drug candidates for further evaluation and will be used to design *in vivo* studies to establish how well they work and their safety as potential medicines.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This Project will benefit humanity in general by helping to accelerate the advancement of therapies to the clinic for unmet medical need, particularly where work is being undertaken on the behalf of clients that lack the necessary facilities to perform *in vivo* testing. It is also anticipated that the discovery of natural substances (Biomarkers) within animal models of disease that are indicative of the progress of that disease may be discovered that are also relevant to humans. This means animal models of disease can be developed that are more relevant, thus causing less animals to be used and less animal suffering. This information will reduce the development time of new therapies and treatments that have the potential	

	to reduce human suffering
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (normal or GA) 23,000 Rat (normal or GA) 12,500 Guinea pigs 1,800
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?	In an attempt to reduce animal suffering, protocols have been designed to have the lowest severity limits possible. To improve animal welfare, the following considerations will be met; where possible animals will be group housed with extensive use of environmental enrichment, surgical techniques will only be used where there are no alternatives and if used, appropriate analgesics will be given and all protocols will be assigned humane endpoints. Most of the animals used on this Project will experience a single injection/oral dose followed by a series of blood samples, typically over a 24 hour period via a temporary cannula or directly via venepuncture of the tail vein, If tissues are required, then animals will be terminally anaesthetised and tissues and or blood will be taken. Minor, transitory discomfort is anticipated upon injection/dosing. Adverse effects due to the test substance are anticipated to be rare. Any animals exhibiting continued distress (after the transitory discomfort of dosing), will be humanely culled. A small percentage of animals used may be surgically cannulated but this is anticipated to be very small, if at all, depending on client requirements. All surgical interventions will be performed under general anaesthesia. Systemic antibiotics may be given prior to, during or after surgery on veterinary advice. Pro and post-operative analgesics will be used as advised by a veterinary surgeon. Once cannulated, the animals will typically receive I dose of test substance which will involve the same degree of discomfort as previously described and blood or bile will usually be sampled over a 24 hour period, ending with humane culling. The remaining animals will have terminal procedures performed under general anaesthetic. Protocols will always end in a Home Office approved, humane method of culling.
Application of the 3Rs	
1. Replacement	*In slilco* and *in vitro* approaches are becoming

State why you need to use animals and why you cannot use non-animal alternatives	increasingly powerful tools in the design of drug molecules with optimal properties and are being used extensively throughout Argenta. These approaches can help in understanding various isolated aspects of a molecule's suitability as a medicine and these methods are used to screen out compounds that clearly do not have the desired properties. However, because the suitability of a molecule as a medicine depends on many factors, not just those that can be studied in isolated in vitro experiments, *in vivo* studies need to be conducted to determine the actual properties of a new molecule in the body. Although not identical, small mammals (e.g. mice, rats and guinea pigs), have a similar enough physiology to Man to be able to use these to characterise a potential drug's ADME properties. Species selected for this Project represent the lowest form of vertebrates in which these types of studies can be conducted and are species that will be used for later pharmacological and safety evaluation of drug candidates. *In vivo* studies are required because the multiple processes involved in determining how well a drug is absorbed, where it goes to, how it is metabolised and how it is excreted are difficult to replicate in the in vitro situation. The use of *in vitro* screens prior to in life studies will minimise the numbers of animals used. Also, compounds going into further development will have appropriate pharmacokinetic profiles in those species that are likely to be used in safety assessment studies. This will minimise the use of animals in the later stages of drug development projects by identifying unsuitable compounds at an early stage. Other efforts to reduce animal use include combining PK and efficacy studies and the use of PK samples to investigate markers of disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The PK for a particular compound in a given animal species is fairly reproducible and given that drug or metabolite concentrations are measured rather than efficacy or a safety effect, the number of animals used for a PK study is relatively low (typically 3 per dose route). In-house experience has shown that plasma exposure data obtained following intravenous dosing in rats is very reproducible and so where appropriate and particularly when *in vitro* screens are not predictive of metabolic stability, iv. (n=3) studies will be performed at an early stage to investigate *in vivo* metabolic stability. Data obtained from these

	experiments could negate the need for further work on a compound or a particular structural series of compounds and hence reduce potential animal usage. Reduction has been a focus of attention throughout the protocols of this licence; approaches regularly used include the use of software to simulate multiple dosing PK, rather than repeat dosing animals. Cassette dosing strategies where applicable can provide multiple compound evaluations in one animal and increased assay sensitivity has led to serial sampling in mice where full PK profiles are generated in single animals rather than the multi animal approach utilising 3 animals per timepoint.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Species to be used in this project will be determined by the pharmacology of the compound in question. For pharmacological evaluation it is necessary to use a species whose relevant receptors/biochemical processes give a good model for those in Man. Therefore, in support of pharmacology, pharmacokinetic studies may be performed in a species which may not be a safety assessment species. Additional studies may also be required to support pharmacology and/or biochemistry studies in genetically altered animals. PK studies will be performed in the lowest vertebrate group shown to exhibit particular disease pathologies. Mice will exhibit the relevant asthma and COPD pathologies when exposed to appropriate stimuli. Other species may be used for PK modelling such as rat and guinea pig if these are more appropriate for modelling specific features of these diseases. In order to improve general animal welfare and the scientific integrity of experiments, where possible the following considerations will be met: 1. Animals will be group housed. 2. Group sizes will not exceed ASPA stocking densities. 3. All animals will receive environmental enrichment including a selection of nesting material, refuges/hiding places and foraging. 4. All animals will undergo acclimatisation before use.

Project 6	**The origin of new β-cells in pregnancy**	
Key Words (max. 5 words)	β-cells, pregnancy, transdifferentiation. 4OHT, transgenic	
Expected duration of the project (yrs)	3	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	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)	Determine the origin of new β-cells in mouse pregnancy. Trace cell division and cell transdifferentiation in the adult mouse pancreas during pregnancy. This is currently the subject of an important scientific controversy. Advances in this field may help in the search for a cure for 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)?	1. Find non-β-cell precursors of new β-cells under completely natural circumstances (pregnancy). This is a long-standing puzzle which has aroused widespread interest among researchers. 2. Once the origin of new β-cells is understood, it may become possible to cure, or at least substantially alleviate, the diabetic's need for insulin.	
What species and approximate numbers of animals do you expect to use over what period of time?	9500 transgenic and 240 wild-type mice over a 3 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	It is possible (but not expected) that a small percentage of mice will die as a result of injections of tamoxifen or related substances. It is unknown whether such deaths are caused by the injected substance, or would result in any case, simply because the mouse has some small difficulty in tolerating the injection, independently of what is	

end?	injected. To prevent this death, each mouse will be monitored carefully and animals will be killed if displaying clinical signs that cannot be easily treated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We cannot do experiments on humans. A small amount of data can be gathered after deaths in road traffic accidents, but this data is too rare for convincing scientific results. Computer simulation is not possible, because the fundamental biological processes are not understood. It is not possible to simulate in a Petri dish the complicated and varying physiological conditions of a natural pregnancy.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will start with screening experiments that determine, using only a small number of mice, whether there is any hope of a larger experiment succeeding. It the result is negative, we plan to cancel the larger experiment. We will be using highly inbred strains. As a result, variation between mice is decreased as much as possible. We will therefore be able to make statistically significant deductions from a smaller number of mice.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The experimental system based on transgenic mice, is the unique system that can give an incontrovertible result in this contentious field. Other laboratories around the world, investigating the progenitors of beta cells, use a different strain of transgenic mouse for each potential progenitor cell-type. Our advanced microscope technology allows us to search simultaneously for many different possible progenitors, using a single group of wild type mice. Breeding transgenic mice requires far more animals, because most mice of the wrong genotype need to be discarded. Our design reduces the total number of mice needed in two ways, firstly there are fewer discards, and secondly many different progenitors are searched for simultaneously. Conclusions from the wild-type experiments will give strong evidence, but will not be 100% conclusive. After a successful search using wild-type mice, we will make certain of the result using only one or two types of transgenic mice. The overall result is a substantial decrease in the total number of mice needed to produce the same scientific results. Moreover, most of our wild-type experiments will require only mild regulated procedures, and possibly none at all.

Project 7	**Cancer Pathways in Metabolic Disease**	
Key Words (max. 5 words)	Metabolism, cancer, signalling pathways, therapy	
Expected duration of the project (yrs)	Five	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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 genetic changes which cause cancer, and the downstream consequences of these alterations, may have a wider role to play in metabolic diseases such as obesity and diabetes. This project will investigate how the events leading to cancer regulate cellular metabolism. This may identify new treatment options for patients with metabolic disorders. Furthermore, we know that cancer cells must adapt their energy processes to survive and so we will investigate the potential of targeting these processes as new approaches to treating 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)?	Obesity, insulin resistance and glucose intolerance are characteristic of metabolic syndromes that lead to an increase in the incidence of cardiovascular disease and type II diabetes, as well as liver disease and cancer. These diseases represent the principal burden of morbidity and mortality in European populations. These studies will increase our understanding of how these metabolic diseases occur and identify possible ways to treat these disorders. We will also explore the potential of targeting metabolic changes in cancer cells as novel anti-cancer approaches.	
What species and approximate numbers of animals do you expect to use	We expect to use up to 5,000 mice per year over 5 years for this project. Around 75% of these will not undergo any scientific procedures, but will be used	

over what period of time?	solely for breeding and maintenance of colonies.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will be bred to achieve test subjects which may be predisposed to obesity and diabetes. Animals expected to develop metabolic disease will be closely monitored by highly trained staff and humanely culled when exhibiting symptoms of the disease. Approximately 75% of the mice will not show any adverse effects relating to their breeding and not undergo any procedures except for ear notching for identification and genetic testing. These will be humanely killed when they are no longer required for breeding. Some animals will be given an altered diet such as high fat diet, or administered substances which will change aspects of their metabolism (eg statins and metformin which are agents currently used to treat cardiovascular disease and diabetic patients respectively). All animals on treatment will be closely monitored for changes in body weight and may be blood sampled to monitor changes in their metabolism which should cause only mild handling stress and momentary discomfort. A few animals will have their breathing monitored in specially designed cages where occasionally they may have access to an exercise wheel. In a few animals (<5%) tests to check levels of glucose or insulin may be carried out. A proportion of animals (no more than 10%) will develop cancer because of their genetic makeup or because tumour cells have been implanted and allowed to grow. These will be monitored closely for well established clinical signs such as weight loss, swelling of the abdomen, and development of visible or palpable tumours. Some of these animals will be given anti-cancer treatments and the response to these treatments monitored. Any animal that displays signs of illness such as weight loss of 20%, excessive weight gain, immobility or ruffling of the coat will be humanely killed. At the end of the study all animals will be euthanized and tissues collected at post-mortem to gather as much information from the study as possible.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studying metabolism in the lab has obvious limitations and the true effect on energy metabolism as a result of dietary changes can best be achieved in a living organism. Long term consequence of altered metabolism (ie obesity) is also best

	recapitulated in the living animal. Furthermore, tissues are made up of different cell types which can all contribute to cues influencing the reaction to metabolic stresses which is often difficult to recapitulate in non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use inbred strains of mice which are nearly identical to other in genotype resulting in less variability between animals and allow us to use fewer animals to achieve a statistically significant result. We perform pilot experiments using only a few animals, before scaling up to the appropriate numbers for a full study. Numbers are calculated based on our own experience, published literature from other experts and with advice from our in-house statisticians. Repeated blood sampling and *in vivo* imaging allows us to follow fewer animals and gain greater information over time on responses to changes in metabolism.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The mouse is a mammal and warm-blooded which shares many features of human metabolism not found in other cold-blooded species such as flies and worms. Furthermore with the ease of manipulating the genetics of the mouse, this makes the mouse the lowest and best model organism to understand the genetic changes observed in metabolic diseases. We will use widely applied techniques to study metabolism, primarily using changes to diet (eg high fat diet) which requires less handling of animals; but also exercise and drug treatments (eg streptozotocin to induce diabetes). We may use readily available models of obesity if these are considered to be the most refined model to address that aspect of the study, especially as a great deal is known about their humane endpoints. Regular monitoring of weight/welfare will allow us to complete studies at the earliest endpoint in which we observe a significant result to prevent unnecessary suffering by extending the study period. We will always refer to previous studies for adverse effects of known diet/treatments and when a group is given a treatment for the first time, we will initiate the study with a pilot-sized group (n=3-6) which will be closely monitored before extending to a larger number. Animals are housed in a dedicated facility proactive with environmental enrichment and receive

	anaesthesia and analgesia as appropriate. All animals will be monitored regularly for signs of normal behaviour and will be humanely killed if they exhibit moderate adverse symptoms.

Project 8	**The physiological role of B3 vitamins in energy metabolism**	
Key Words (max. 5 words)	Energy, Vitamin B31 Nicotinamide adenine dinucleotide, mitochondria	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Ageing is associated with declining levels of the molecule NAD+ and a reduction in a cell's ability to produce energy via an organelle called the mitochondria. B3 vitamins and their metabolism may improve age related decline an increase the. effectiveness of exercise. We will develop methods to accurately assess B3 vitamin bioavailability and metabolism to address the key questions: How do B3 vitamins regulate and impact: 1) whole body energy homeostasis and 2) 2) the contribution of skeletal muscle function and energymetabolism to question 1 in adult mice during ageing and examine the interaction with exercise.	
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)?	-Translate supplementation of nutrition with B3 vitamins to human use to prevent or delay ageing muscle loss and age-related decline. -Improve our understanding of ageing biology, the mechanisms that contribute to ageing, and how the interaction with exercise can modify and benefit ageing processes. -Shared results and resources to better understand ageing muscle.	

What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years, we anticipate no more than 13000 mice in total — 5000 animals for scientific protocols and 8000 to breed the genetically altered strains.
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? (1)	Ageing We expect adverse effects to be of moderate severity. The mouse strains used in this project have approximately 85% survivorship at 24 months, A number of reasons exist for the 15% non-survival rate at 24 months including natural causes, tumour burden or acute neurological disease such as stroke. Adverse events include: -deterioration of body condition such as skin sores for which may be resolved with analgesic creams. - Increased barbering or repetitive behaviours for which further enrichment actives and conditions will be used. -Overgrown teeth will be clipped if possible. If these conditions are not resolved the animal will be humanely culled if palpable tumours manifest, there is weight loss of up to 20%, or continued piloerection, obvious pain or discomfort, rapid loss of fur condition and general malaise beyond that expected for an aged mouse, intermittent hunched posture or reduced activity. Breeding of GA animals- The lines we propose to use are only expected to experience adverse effects of mild severity. H6PDH mice show age-related muscle function decline beyond 6 months of age classified as moderate severity. We do not intend to use H6PDH mice beyond 6 months. However, if H6PDH mice have phenotypic presentation before 6 months the animal will be humanely culled. Acute and endurance exercise -We expect adverse effects to be of mild severity. Exercise of moderate intensity and duration may induce mild physiological and psychological stress, mitigated by habituation and acclimatisation of the mouse to the environmental conditions of the exercise. Animals will be monitored continuously for indications of injury for which the exercise will be stopped. If the animal fails to recover from any apparent injury, either as a continued obvious impediment to movement, weight loss up to 20% then the animal will be humanly culled by a schedule I method. Fatigue may present as signs of mild agitation and a failure to maintain momentum while attempting to find rest. If this occurs a gentle air blower will activate to encourage completion of the exercise. If the blower activates three times the exercise will cease and the animal placed back in its home cage and monitored for

	adverse signs. These include weight loss of up to 10%, and/or continued piloerection, intermittent hunched posture and/or reduced activity for a period of 24hrs beyond which the animal will be killed by a schedule I method.
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? (2)	Metabolic studies - Altered energy and supplementation diets- Only mild adverse effects are anticipated. Bodyweight and food intake will be closely monitored for signs of ill health and behavioural abnormality, though this is not anticipated. In some cases excessive grooming may occur resulting in skin irritation. If skin becomes broken and inflamed, and is not resolved with topical analgesic cream and monitoring the animal will be humanely culled. Substance administration- The substances to be used are: circulating factors, synthetic analogues, antagonists, heavy isotope metabolic tracers or fluorescent dyes. These have previously demonstrated not to have any adverse effects from administration and consider any adverse effects will be mild, However if animals display signs of toxicity such as reduced mobility and up to 10% weight loss mice will be humanely culled. Animals in all protocols will be humanely killed with or without having blood removed from a major vessel or the heart, which will only be performed under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	No in vitro or alternate system exists to accurately model the complex interaction of factors determining energy metabolism and its interaction with exercise and ageing. While skeletal muscle cells can be cultured and examined to answer specific questions, they cannot represent whole organism responses that may be age-dependent. Ageing is a gradual and chronic process that cannot be modelled accurately, as yet, in any other system that will model human ageing with high fidelity. Animal experiments will solve mechanistic problems that small clinical samples are incapable of solving.
2. Reduction Explain how you will assure the use of minimum	Statistical analysis and pilot studies will ensure that we use the minimum number of mice per group while maximising data quality. We will use strain and age-

numbers of animals	matching, using multiple outcomes in the same animal when possible. To maximise the information gained from a single animal we aim to take perform multiple in vitro analyses. Where possible, cell line work and in vitro manipulations have been designed to yield the maximum possible information and reduce animal use.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mouse genetics and genome manipulation is advanced with a wealth of understanding of murine physiology. The mouse has been selected for this work because: (1) established and reliable transgene technology to test the objectives and ageing parameters and reproducible exercise Mice are not aged beyond that which is scientifically useful. Mice will have access to enriched environments such as running wheels and the exercise interventions are refined to preclude exhaustive or forced methods. Pilot studies and habituation methodologies will be used as required. Mice will be monitored constantly during exercise protocols and at least 2-3 times a week during ageing to assess body condition; this will be on top of daily checks by BMSU staff. All new genetically altered strains of mice to be examined will be closely assessed for undetermined phenotypes that may present through the course of experiments.

<table>
<tr><td>Project 9</td><td>Circadian control of behaviour and metabolism</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Obesity, diabetes, clock, biological rhythms, physiology</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>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>The aim of this project is to further our understanding of the physiological and neural circuits which govern metabolism and energy balance. This work will also examine specifically how our body clocks (the circadian system) contribute to the regulation of metabolism and energy balance, determine the extent to which clock disruption may contribute to metabolic disease.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This work looks directly at the pathophysiology of metabolic disease. At present we have few successful or effective treatment options for patients. Therefore, the potential benefits of this research are high.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approx. 14000 mice (including breeding) over 5 years</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>The majority of the research falls into the mild category, being confined to breeding, routine monitoring of behaviour, and environmental manipulations (diet, light, and ambient</td></tr>
</table>

level of severity? What will happen to the animals at the end?	temperature). Studies in the moderate category include the surgical implantation of physiological monitoring devices, slow release pumps, and other surgical techniques such as intraneural injection and removal of the adrenal glands. The animals recover well from these procedures, with adverse effects limited to the immediate postoperative recovery. As we are studying normal physiological and behavioural processes that govern circadian timing and metabolism, it is critical for our work that the animals are as healthy as possible. As soon as the scientific objectives have been reached, animals will be killed by an accepted and humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Many tissue and organ systems contribute to energy homeostasis, metabolism, and circadian rhythmicity, and therefore the pathways and events involved cannot be modelled within an in vitro cell or tissue culture setting. In-vitro assays do however offer a powerful model to test how genetic alteration of clock function may impact on the core circadian oscillator, the re-setting characteristics of this oscillator to biochemical stimuli, and the role of metabolic-acting drugs and compounds on clock function. By using lines of genetically modified mice expressing a clock gene reporter, it will be possible to define key responses using an in-vitro model and use these data to inform the design of subsequent in-vivo experiments. Whenever possible stable cell lines will be used to investigate clock and metabolic coupling (for example in 3T3-Li adipocytes and rhythms in lipid metabolism).
2. Reduction Explain how you will assure the use of minimum numbers of animals	To ensure that we use the minimal number of animals, careful consideration is given to experimental design. Based on our experience of the animal models and techniques used, we use power analysis calculations to determine the minimum number of animals required. Generally, group sizes of 6-8 are required to achieve 80% power assuming an effect size of 30-50%. Simultaneous recording of multiple physiological responses offers a significant reduction to the number of animals used, as well as increased statistical power. Novel technology for in vivo

	recording of gene transcription significantly reduces animal use, through lessening the need for cross-sectional sampling. Whenever possible stable cell lines will be used. In particular, use of circadian clock-reporter lines allow extensive use of in-vitro assays on isolated cells and tissue (e.g. to test the effect of clock acting drugs prior to any application in vivo).
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Our studies focus on mice. Mice offer unparalleled opportunities for investigations of the underlying genetic mechanisms involved in circadian timing and physiological function. Many pathways involved in normal regulation of metabolism, as well as the pathological events associated with obesity are well conserved between mice and humans. The principal mechanism for ensuing minimal welfare cost is close monitoring of the animals, understanding the normal physiology of the animals, and optimal design of all animal experiments regardless of expected severity.

Project 10	**Embryogenesis, stem cells and cell fate decisions**	
Key Words (max. 5 words)	Stem cells, cell fate, embryogenesis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	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)	Many cells during embryonic development, but notably also stem cells in the adult animal, have alternate fates where they can stay unspecialised, adopt one or more mature (differentiated) and functional states, or die. This project is concerned with how these decisions are made, and to do so we focus on the role of specific genes and genetic pathways in both normal situations and situations when these go awry, such as with congenital and chromosomal abnormalities, physiological stress and trauma, and in cancer and ageing. It follows that understanding the underlying mechanisms for cell fate decisions, which may then be controlled as part of a therapeutic strategy, is of fundamental and clinical importance. The main systems we study are the early embryo, the central nervous system, some sensory systems such as the inner ear, the reproductive system (especially the gonads), the pituitary, and the gut. These are all systems where cell fate decisions involving progenitor cell types and/or stem cells are critical, amenable to investigation, and have both biological and clinical relevance. As part of our work we also study specific types of gene that are known or suspected of being important for cell fate decisions and the biology of stem cells. The objective in this case is to determine how the genes and their products (such as transcription	

	factors or components of a signalling pathway) work at a molecular level.
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 main reason for this work is to provide basic understanding of fundamental mechanisms. However, knowledge of how decisions of cell fate are reached, including the genes involved, and the properties of stem cells within these systems, will ultimately allow them to be controlled or introduced in a beneficial way for treatment of human and animal disease and trauma. Our project is also likely to benefit diagnosis of genetic disease, to inform clinical treatment, and ultimately to increase the range of options available for treatment. These statements are based on our track record. For example, genes that we discovered and/or studied such as *SRY*, *SOX9*, *AMH*, *DAX1*, *SOX3*, *FOXL2*, are now routinely examined to diagnose the underlying cause of disorders of sex differentiation, where this knowledge helps counsel patients and inform clinical care. AMH, which we first described as being expressed in the postnatal ovary in mice, is now routinely used to determine ovarian function in assisted conception. *SOX2* and *SOX3* are now screened in disorders affecting CNS, pituitary and sensory system development, and it was our work that directly led to patients with anopthalmia due to mutations in *SOX2* now being managed for the pituitary defects that always accompanies the eye problems. *SOX2*, which our work showed was necessary for pluripotency, is one of the critical genes used to derive patient specific iPS cells, which are just beginning to be used in trials to treat, for example, macular degeneration. *SOX2*, *SOX4* and *SOX9* are also beginning to be used diagnostically in some forms of cancer where their overexpression is correlated with prognosis.
What species and approximate numbers of animals do you expect to use over what period of time?	We mostly work with mice because of the powerful techniques and knowledge available for this species in terms of genetics, embryology, cell biology and behaviour, but also as they have relevance to the human situation. Over the last 5 years we used a total of approximately 40,000 and we anticipate similar numbers will be used during the 5-year duration of this PPL. For some types of experiment we may use rats (probably no more than a few hundred), because their larger brain size and more complex behaviour is more appropriate for the types

	of manipulation required; for example studies on stem cells and stroke and methods to induce repair. We may occasionally study the opossum (fewer than 250), a marsupial, either because their early birth (compared to that of rodents) permits access to and observation of specific tissues, or to give information about mammalian evolution. We also study lower vertebrates, notably chick (fewer than 10,000 embryos), frog (Xenopus; less than 1000), and fish (zebrafish; 3000), where these can give critical evolutionary insight, have specific experimental advantages, or when work in mammals is not necessary. (N.B. All figures given are the maximum numbers of animals to be used over a 5-year period. Moreover, many of the experiments are on fetal forms).
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 vast majority of our regulated experiments are of the mildest severity and concern the breeding and observation of genetically altered mice and/or minimally invasive procedures such as administration of substances by injection or the killing of embryos or adults. Adverse effects are neither expected nor seen in all but a very few of these cases. A much smaller number of protocols are of moderate severity. These involve surgical procedures (which are carried out under anaesthesia and with analgesics for pain relief), and/or the generation and study of genetically altered mice with, for example, congenital abnormalities, or which are likely to develop tumours. There will always be a few animals that die suddenly and unpredictably for no known reason. A few procedures have an increased risk of unexpected and sudden death, but this will still affect only a very small minority of animals to be used in this project (fewer than 0.1%). Any animal approaching severity limits listed for a protocol will be killed, and all animals subject to a procedure will eventually be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Most, if not all cell fate decisions in the embryo and adult animal take place within a complex environment, where events intrinsic to the cells are influenced by a variety of extrinsic signals. The latter can involve molecules that can act locally or over considerable distances (such as growth factors, cytokines and hormones), and which may originate from neighbouring cells, or from anywhere within the body (or even be from the external environment).

	Moreover, most tissues develop in a complex way in three dimensions over time in a carefully orchestrated manner, and require vasculature and innervation to operate. Therefore, although some aspects of certain cell fate decisions can be studied in vitro, and we both use and develop such approaches, it is generally essential to study them in animals (as a minimum to judge the suitability of in vitro systems to give meaningful information). This is particularly true of the complex systems and processes we investigate.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our specific experiments are designed to use the minimum number of animals required to give robust answers, making use of statistical methods (such as power calculations) where appropriate. We test methods and reagents in vitro whenever possible prior to their use in animals. We will use in vivo imaging when feasible, which allows information to be gained over time from single animals. We will use efficient methods to generate and maintain genetically altered animals, and make use of sperm and embryo freezing to avoid having to keep strains as live animals when they are not actively being studied.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We choose well-established protocols, known to have minimal harmful effects, whenever possible. We also choose to work on lower species of vertebrates (such as chick instead of mouse) if we know that they will give comparable information. Whenever practical, we prefer to make genetic alterations that are inducible, so that the animals do not show a phenotype until expression of the candidate gene or a deletion is induced. When the experiment is predicted to lead to harmful effects outside the body system under study, we will provide treatments designed to alleviate these.

Project 11	**Extracellular regulation of insulin resistance**	
Key Words (max. 5 words)	Insulin; Obesity; Type 2 Diabetes.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	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 hormone insulin promotes the absorption of glucose from the blood to skeletal muscles and fat tissue. Type 2 Diabetic patients often have a defect in responding to insulin ("insulin resistance") and fail to maintain normal stable glucose levels in the blood. Given the high and increasing prevalence of Type 2 Diabetes and its associated social and economic burden on the NHS and society, understanding how insulin resistance develops and how we can target it to develop cost-effective therapies are timely and extremely important goals. Conventionally, research into insulin resistance has mainly focused on studying the direct action of insulin on molecular pathways INSIDE the muscle cells. Our recent work has shown that the proteins and molecules OUTSIDE the muscle cells also undergo changes during the development of insulin resistance. Furthermore, when we removed one of these molecules in mice, we found that they had an improved ability to respond to insulin, especially in the skeletal muscle. This finding opens up an entirely new area of diabetes research and may prove more specific to diabetes than trying to target the inside of the muscle cells. In the current study, we aim to study the effects of such changes outside the cell on the development of insulin resistance in much more detail.	
What are the potential benefits	The proposed research will provide significant	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	benefits to other researchers in the field of obesity, insulin resistance, and metabolic disorders. The proposed study may generate new insights into how hormones like insulin actually work. These might, in future, lead to new and effective treatments for diabetes.
What species and approximate numbers of animals do you expect to use over what period of time?	Genetically altered mice will be studied in this proposal. Approximately 2000 mice will be bred, of which 500 might undergo further regulated procedures, 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?	The genetic alterations themselves are not expected to cause adverse effects but may influence whether the mice become diabetic (e.g., after being fed a high-fat diet). Diabetes in mice is often first suspected when the animals' water intake (and output of urine) increases. It can then be confirmed by a blood test. Close observation of the clinical condition and body weight will then ensure that animals are killed humanely while these deviations from normal welfare are still moderate. Tissues will be collected post mortem for further laboratory analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mechanisms by which animals (including humans) regulate their body weight, glucose levels and fuel storage/energy expenditure are complex and involve the interplay of multiple organs (e.g. muscle, liver and fat) and pathways. These cannot easily be mimicked in non-animal systems. Mice have been chosen for our studies because this species has been extensively used as a model organism in the understanding of human metabolic diseases. Importantly, gene targeting technology and dietary manipulation is widely available for the mouse and thus allows investigators to precisely establish the relationships between genes and biological processes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculation (alpha=0.05; Power=0.8) estimates that 8 animals per group would be needed to detect 40% difference in insulin sensitivity by the clamp experiments. Statistical analyses will be performed using either Student's t test or two-way ANOVA followed by Tukey's post hoc tests as appropriate. The significance level will be at $p<0.05$.
3. Refinement Explain the choice of species	Many of our studies will involve the collection of blood samples from a mouse over a period of time. Rather

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.	than stress the animal unduly (which would also interfere with the quality of the scientific information), we shall implant sterile catheters into important blood vessels, under general anaesthetic. Once the animals have recovered completely from this surgical procedure, we shall then be able to take small blood samples without causing undue stress.

Project 12	**The mechanisms of ageing and age-related disease**	
Key Words (max. 5 words)	Ageing, age-related disease, healthspan	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	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)	Ageing is the major risk factor for a number of common human diseases such as cancer, dementia, diabetes and osteoporosis. Recent research has shown in a range of animals including mammals that the ageing process itself can be delayed. This in turn is associated with a reduction in age-related disease. These findings have been shown across most species including rodents and primates and suggest that if we can identify ways of delaying the ageing process with drug treatments then we will be able to treat some of the diseases of ageing in humans. Our own studies have previously identified two of the signalling processes that regulate ageing in mice and shown that alteration of these processes can delay ageing and ameliorate the diseases of age. However, these processes are complex and involve a wide range of molecules and tissues. The objective of the current project is to define the how these molecules control ageing by manipulating them in different tissues and by making specific alterations in the genes that act downstream of these signals. These studies will be performed in mice, which will be studied as they age and examined for resistance to age-related disease. These studies will therefore increase our understanding of the molecular basis of ageing.	

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)?	Our findings on ageing have started to reveal potential therapeutic strategies for the diseases of ageing. However, the mechanisms we have identified to date may not be ideal drug targets. Our current project will refine our understanding of these mechanisms with a view to identifying potential therapeutic approaches to ageing related diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	These studies will use mice and will use approximately 3500 mice per year and the studies will last 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The overall severity is moderate. For the studies on lifespan, mice will develop the features of normal ageing. For the studies on metabolism and imaging during ageing, animals will have injections and blood sampling which rarely lead themselves to adverse effects and are performed using best current practice. Other expected adverse effects for these studies are those associated with normal ageing. For the studies on behaviour in old animals there are no expected adverse effects other than those associated with normal ageing. For all procedures animals will either die naturally or be culled by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The study of mammalian ageing requires the use of animals because ageing is feature of intact organisms and cannot be adequately modelled in non-animal alternatives. The assessment of lifespan therefore requires the study of whole animals. When studying the diseases of ageing it is also necessary to study intact animals because these diseases develop within the context of the ageing process. We also wish to study cells and tissues from animals as they age to gain insights into the effects of the ageing process. While non-animal alternatives cannot be used our choice of animals to study has been strongly guided by studies in flies and worms. Our previous ageing studies in mice have studied the same genes and pathways that were found to extend lifespan in flies and worms and we have adopted this approach again. While not replacing the use of mice it gives strong evidence that we are studying the correct types of pathways thereby reducing overall animal usage.
2. Reduction	The numbers of mice to be used are based upon our

Explain how you will assure the use of minimum numbers of animals	calculations using our previous published data on ageing and age-related disease and will be the minimum numbers of mice required to identify mice that are long-lived and protected from the diseases of ageing. Our breeding programme and experimental designs will be streamlined and will employ where possible longitudinal studies on the same mice (including non-invasive studies) which will reduce the overall numbers of mice required to reach the scientific end-points.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Our studies will be performed in mice. They are the lowest mammalian vertebrate group with the necessary characteristics in which ageing and ageing related diseases have been characterised. The mouse is the primary mammalian species in which gene manipulation is undertaken thus permitting the generation of genetic models for study. Mice are also well suited for use in longevity studies because of their relatively short lifespan and small size which both carry economic and practical benefits. There is a wealth of pre-existing information about ageing in mice and they are a well-established model for testing genetic interventions that might alter lifespan and the diseases of ageing. Mouse models have proved in many cases to be excellent models for the understanding of both human physiology and disease. To minimize harm to animals we have established and validated in our previous work monitoring protocols for mice as they age which identify at an early stage any potential welfare costs and allow us to intervene to minimize adverse effects. All animals will be housed in groups where possible with appropriate environmental enrichment and husbandry undertaken according to current 'best practice' at our Institution.

Project 13	**Endocrine action and endocrine cancer therapy**	
Key Words (max. 5 words)	Androgens, prostate, cancer, endocrine	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✔	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)	Prostate cancer (PC) is the most prevalent malignancy in western males. Initially prostate cancer growth depends on circulating androgens (the predominant sex steroids in males, responsible for masculinisation during development and at puberty), however relapse and resistance to treatments invariably occurs as tumour progress to refractory, 'castrate resistant' prostate cancer (CRPC). Resistance to hormonal therapy is the major cause of death in these patients. Inhibiting androgen signalling, while an effective therapy initially, has wide-reaching implications, because androgens have vital roles in a number of different organs in both males and females. The targets of androgens are not limited to the prostate and other male sex organs (such as the testis, seminal vesicles), but also include bone, the central nervous system, adipose tissue, hair follicles and ovary. Therapies that inhibit androgen signalling can have serious side effects, also including effects on fertility. We therefore aim to study and assess side effects of hormone therapies used in the clinic, and new drugs in development, using a novel genetically-modified mouse model expressing a "reporter" gene, which causes a luminescent signal to be emitted when androgen signalling is activated in a cell . In this way androgen signalling activity can be assayed in all organs, including but not limited to the prostate, by live	

	imaging. Moreover, by crossing these androgen reporter luciferase mice with other strains of transgenic mice, for instance that develop prostate cancer, we can study the role of androgen signalling activity during the progression of the disease and investigate the efficacy of the treatment in these novel models. Other mouse models we use carry human prostate tumours, and will allow the same therapies to be tested on these in a setting that mimics the situation in a human patient. The androgen signal is transmitted by a specific receptor, the Androgen Receptor. The Androgen Receptor (AR) is part of a large family of important proteins called Nuclear Receptors, and some others have also been implicated in prostate cancer. In parallel with what is described above, we aim also to study the effects of targeting other nuclear receptors which have been implicated in prostate cancer and other cancers. Emerging evidence indicates there is likely to be cross-talk between other nuclear receptors and the androgen receptor, so a combination of therapies may be effective.
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)?	In this project we aim to evaluate therapeutic agents for prostate cancer, both novel and already approved, in order to assess their side effects. These results will be published in peer reviewed journals and therefore bring new scientific knowledge to other researchers. In the long term the benefits will be the development of more targeted drugs with reduced side effects, and consequently a better quality of life for the patients. We will also establish new collaborations with other research institutes and companies, in order to test and evaluate the compounds currently in development and assist our collaborators with their drug discovery programs. During the length of the project we also aim to investigate mechanisms of resistance and/or cross talk between nuclear receptors. The results will increase the scientific knowledge in the field and will allow us to identify new targets or pathways involved in disease progression. Since we know that a number of the nuclear receptors under investigation here have a role in normal androgen production and fertility, the effects

	of treatment with drugs that target such nuclear receptors will also allow us to further understand the implications of receptor modulation on fertility and normal hormonal function. In the long term this will lead to better treatment and quality of life for patients. Overall, the studies under this project will drive clinical development of the drugs, ultimately towards the evaluation and design of successful treatments for patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 6500 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?	The severity limit of the procedures will be mild and moderate. We will reduce the stress to the animals as much as possible with the use of imaging and non-invasive techniques. Some mice will be used for breeding and some might be injected with human cells to develop tumours. We will closely assess the tolerability of the injected compounds by monitoring the body weight.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	During this project we aim to elucidate where and when the androgen signalling pathway is active in the whole animal, in which organs drugs aimed at this pathway can block this, and also how these affect prostate tumour growth in a genetic model of the disease. Cells grown in the lab are not complete models for cancer because they lack the context (e.g. other organs, blood vessels) in which tumours develop. This can only be recapitulated in a whole animal, necessitating use of mouse models. These also allow us to study the mechansims by which cancer spreads around the body Further, preclinical testing in animal models is a prerequisite for drug discovery, before therapeutics can enter phase I testing. For the potential therapeutics, we need to demonstrate that effects on cancer cell growth are reproducible in an in vivo setting. Another important consideration is off-

	target effects on other organs, the major focus of this study. These cannot be predicted using a single cell type, but requires a mammalian model system. Nevertheless, in vivo assessment is only carried out following rigorous testing of potential therapy targets and/or drugs using in vitro cell culture model systems. In fact in vitro cell based screening of targets/compounds for mechanisms of action and efficacy, as well as in vitro pharmacology studies for compounds are carried out in order to limit compounds/targets to only the most appropriate and well-defined targets/compounds.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In vitro screening also helps to ensure that the fewest possible number of compounds/targets are taken forward to animal studies and hence reduces the number of animals used. In designing each experiment, we consult a statistician for advice on the minimum number of animals required to ensure a statistically valid result. The use of the nude mouse model (mice lacking a functional immune system,, so able to carry human-derived tumours) in the generation of hormone dependent tumours is well established. The system is robust and tumour establishment is efficient, thereby minimising the numbers of animals that need to be used. Moreover, our genetically modified model, expressing a luminescent signal in response to androgen signalling, will allow us to use non-invasive imaging to monitor drug response and androgen receptor activation/repression. This technique allows the sequential imaging of the mice, greatly reducing the numbers of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Apart from the tumour initial implantation, the light emission imaging and the tumour growth measurements are non-intrusive/non-invasive. Some of proposed models result in tumour growth only at the site of inoculation and others may feature tumour spread. As such, the model is not severe and is well tolerated by the tumour bearing animals. Moreover using the genetically modified reporter mouse model we will be able to visualise the activity of the androgen receptor pathway in response to different

	drugs but also in the disease setting. To our knowledge, there are no equivalent models to compare these systems to. Importantly, *in vivo* imaging minimises numbers as we can visualize tumour growth and treatment effects by imaging the same animals repeatedly without need for sacrifice. During breeding, animal numbers will be kept to the minimum required to generate the model and provide sufficient numbers for proposed experiments. Careful monitoring of health and well-being, together with regular recording of the weight, will be utilised to predict adverse side effects and ensure humane endpoints to experiments. Anaesthesia and analgesia are used wherever appropriate. Whenever necessary/appropriate, the advice of the named veterinary surgeon will be sought in order to determine when anaesthesia and/or analgesia might be required.