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>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">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>
</table>

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 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.

<table>
<tr><td>PROJECT 5</td><td>Engineering Immunity to Cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Genetic modification, T-cells, Cancer, Therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[6]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[7]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Cancer is currently the second most common cause of death in the UK after heart disease and is predicted to become the most common cause of death in the UK in the next 20 years. Therefore, despite recent advances in the treatment of cancer, there is still an urgent need for the development of better anti-cancer therapies. In the last 10 years it has been demonstrated that a cancer patient’s immune cells can be genetically engineered to recognise and kill their cancer cells but not normal non-cancerous cells. The testing of genetically engineered immune cell therapy in cancer patients has shown that it can lead to total cancer eradication in some patients, but other patients have not responded so well to this treatment. While this approach to treating cancer shows great promise, a number of hurdles need to be overcome</td></tr>
</table>

	for it to become a widely available anti-cancer therapy. Chief amongst these are the need for the further development of genetically engineered immune cell therapy so that: (i) it can be used to treat a greater range and number of cancer patients; (ii) it will be more effective at completely eradicating cancer in treated patients. Therefore, the objectives of this project are: (i) The further development of genetically engineered immune cell therapy to enable it to be used to treat a greater number of cancer patients; (ii) To identify ways of enhancing the anti-cancer efficacy of engineered immune cell therapy so that it is a more effective therapy in treated patients.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary benefit of this project will be the further development of genetically engineered immune cell therapy, thereby enabling a greater number of cancer patients to be treated with this promising therapy. Furthermore, this project is expected to lead to the identification of strategies that can be used to enhance the anti-cancer efficacy of genetically engineered immune cell therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will only use mice that have been specifically bred for research purposes. We estimate that we will use up to 960 mice per year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	In addition to normal mice, we will use some genetically altered strains of mice. For example, we will use mice that lack their own immune system as this enables us to introduce a human immune system into these mice The breeding of the genetically altered mice used in this project results in minimal side effects and is classified as being of a mild severity. In this project the experiments we will carry out will involve some of the following procedures: (i) Injecting cancer cells, immune cells and other therapeutic agents into mice using a needle. These procedures will induce some stress due to restraint and transient discomfort from needle insertion (ii) Occasional blood sampling using a needle. This procedure will induce some stress due to restraint and transient discomfort from

	needle insertion. (iii) Limited does of radiation of mice to partially deplete their own immune cells prior to the transfer of cancer-targeted genetically engineered immune cells. High doses of radiation can cause sickness in mice but we will use lower doses that are very well tolerated. In addition to the adverse effects described above, the primary expected adverse effect associated with these experiments is cancer cell growth, which left unchecked will prove fatal. Furthermore, there is the possibility of autoimmune reactions due to genetically engineered immune cells attacking normal non-cancerous cells. Therefore, any mouse showing signs of distress or pain reaching a moderate severity level will be humanely euthanized by an approved method. Finally, at the end of experiments mice will be humanely euthanized by an approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Prior to being tested in mice all of the genetically engineered immune cell therapies are tested *in vitro* (i.e. not in animals) to determine if they are able to recognise and kill cancer cells safely and effectively. However, to provide effective anti-cancer therapy genetically engineered immune cells must: (i) survive and expand after transfer into a patient; (ii) circulate through blood and migrate out of blood vessels into the cancer tissue; (iii) migrate through the three dimensional structure of the cancer and kill cancer cells. These complex processes cannot be adequately modelled *in vitro.* Furthermore, well-designed mouse studies can be used to identify unpredictable side-effects that may arise as a result of immune cells killing normal non-cancerous cells. Finally, *in vitro* systems cannot adequately model the complex web of interactions that occur between transferred engineered immune cells and other cell types within the body.
2. Reduction Explain how you will assure the use of minimum numbers	Appropriate statistical analysis will be carried out before experiments to determine the minimum number of mice required for an informative answer.

of animals	Where necessary, we will use pilot studies with small numbers of mice to help determine the minimum number of mice required to reliably obtain an informative answer to the experimental question. By using imaging technologies to visualise both cancer cells and immune cells at different time points in individual mice we can obtain more data from each mouse in an experiment and thereby reduce the total number of mice used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice have the lowest neurophysiological sensitivity among potentially suitable animal species. Mouse and human immune systems are very similar and the mouse immune system is arguably the best characterized amongst vertebrates. Furthermore, the reagents and tools required for the studies described here have been designed for use in mice. All experiments will be conducted in accordance with the UKCCR guidelines on the welfare of animals in cancer research and we will follow laboratory animal science association (LASA) guidelines. In addition, most of the mouse models and protocols that will be used in this programme of work are well established in our laboratory or those of our collaborators.

Project 3	**Immuno-modulatory and Inflammation Research**	
Key Words (max. 5 words)	autoimmune disease Immune system, modulation	
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
	Yes	Translational and applied research
	Yes	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)	Auto-immune diseases, such as rheumatoid arthritis, some cancers and inflammatory bowel disease are caused by the immune system attacking its own body. It is thought that common biological mechanism(s) cause these and other auto-immune diseases. New medicines are required because the treatment of patients with these types of diseases is currently unsatisfactory with only some symptoms being reduced but not permanently cured. The primary aim of this programme of work is to identify new treatments for human diseases. Identification of novel therapies which are effective in animal models of immuno-modulation and inflammation and identifying new pathways involved in immuno-modulation and inflammation diseases will lead to the development of medicines to treat such human diseases. Although assessment of potential new medicines in test-tubes will provide valuable information, we need to follow the effects in the whole animal to ensure	

	that the immune system is responding as predicted.
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 overall benefits of the work are to improve the understanding of auto-immune diseases and to develop improved medicines for patients with auto-immune diseases. Diseases such as Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Inflammatory Bowel Disease (IBD), Cancer and Psoriasis are major diseases within this category and affect a large proportion of the human population worldwide. RA affects between 0.5 and 1% of adults in the developed world. Cancers as a group account for approximately 13% of all deaths each year. These diseases not only affect the patients' lives but also those of their families and carers and potentially overload the health care systems. Currently there are treatments that may reduce some symptoms of these diseases in some small percentage of patients, but none work in all patients. Results from studies performed under this licence will provide key information on the likelihood of a particular new medicine working in these types of diseases
What species and approximate numbers of animals do you expect to use over what period of time?	Up to a maximum of 11,250 rats and mice over a period of 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We need to study parts of the immune system as it works in the whole animal. Some animals will have their immune system stimulated and the acute effects of this will be studied. In other studies we will induce states similar to early rheumatoid arthritis, cancer and inflammatory bowel disease (IBD). This may result in swelling of the limbs or production of cancerous lumps or symptoms of IBD such as weight loss, diarrhoea or bloody faeces in some animals. We will take blood samples, measure markers of the response and determine whether new medicines can affect the response. Some animals will experience discomfort. This will be monitored. Any animal displaying discomfort and distress beyond the minimum required to achieve the aims of the study (e.g. piloerection, hunched posture, subdued

	responsiveness, weight loss >20%) will be killed humanely. Anaesthetics and analgesics will be used to reduce the discomfort induced by any surgical procedure. The use of analgesics may impact the disease progression and interfere with the outcome of potential new medicines being tested. Carprofen is routinely administered prior to and for one day post surgery as an analgesic. This substance is a non-steroidal anti-inflammatory agent and hence its long term use may interfere with the biological systems involved in these studies and may inadvertently affect the study outcomes. Rats and mice with arthritis will display swelling of the limbs however, they are expected to still be able to move around their cage, interact with cage mates and display normal feeding and grooming behaviours. Should any animal not be able to display these behaviours it will be killed humanely.. The investigation of the new medicines should reduce any suffering experienced by the animals. At the end of studies the animals will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All work using animals will be preceded by studies using human and/or animal isolated blood, organs, tissues or cell lines. The immune systems of rodents and humans are very complex with many different parts working at different times and in different ways sometimes together sometimes alone. We can study parts of the immune system in test-tubes but cannot study the immune system as a whole, so we have to use animals with an immune system like humans. The immune system of rodents has been extensively studied, and is sufficiently similar to human to provide appropriate models for this work.
2. Reduction Explain how you will assure	The estimated number of animals is based on our previous experience of designing these types of studies. For new study designs we will consult with a

the use of minimum numbers of animals	statistician to ensure that we are using the minimum number of animals to achieve the objectives.
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 will read the scientific literature and document how the most appropriate design for the aim of our studies will be chosen. Challenges to the immune system, the volumes of injections and the number of blood samples taken will be balanced limited to ensure that the welfare of the animal is not compromised and that the level of discomfort is kept to a minimum, while achieving the scientific need for these studies. Animals will be routinely group housed with appropriate litter, nesting material and environmental enrichment. A veterinary specialist will be available to advise on care for the animals and can be contacted outside normal working hours if necessary.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>The biological significance of DNA methylation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Rett Syndrome, epigenetics, mouse models</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td>Yes</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Without changing the basic sequence of DNA it can still be chemically altered in ways which effect whether or not a particular gene is expressed. We would like a greater understanding of the function of one such chemical modification of DNA; methylation. Our research will focus on how DNA acquires this signal and in turn how cells then read and act upon this signal.</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>Defects in genes involved in the interpretation of methylation are associated with human diseases such as cancer and Rett Syndrome. Similar work in our lab has already established that Rett Syndrome is potentially curable. A better understanding of the underlying biology of such diseases will improve future therapeutic approaches.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice
4750 per annum</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?</td><td>Rett syndrome is a disabling neurological disorder which primarily affects girls. It is known that mice with genetically altered copies of the Mecp2 gene develop clinical signs similar to Rett syndrome, after several weeks. The resultant severity in mice is moderate. The majority of the animals generated will be on breeding protocols, to maintain the line, with nothing happening to them. Animals will not be allowed to develop clinical signs unless this state is specifically required for an experiment. Some animals will develop signs such as impaired mobility. A minority of animals will be administered</td></tr>
</table>

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

	prospective therapeutic substances via injections into the brain. This is done under anaesthesia and using adequate analgesia to limit post-operative pain as advised by a veterinary surgeon. All animals will be euthanized using authorised procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We wish to study the consequences of the proposed genetic alterations in organs such as the brain. The complex biology of such multi-cellular organs cannot be modelled *in vitro*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful consideration will be given to all breeding strategies to ensure we can produce sufficient animals with the appropriate genetic status in the most efficient way, thereby reducing the numbers of animals produced overall. When novel approaches are being tested, we will conduct pilot studies with smaller groups of animals prior to further experiments. Careful experimental design and statistical analyses will enable us to determine the smallest number of animals required to give us meaningful results.
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 offers the best system in which to study the consequences of changes to genes such as Mecp2 as it provides us with the best characterised model of the human disorder, Rett Syndrome. Mice will not be allowed to develop clinical signs unless specifically required for an experiment and in all experiments use of early humane end-points is implemented using clearly defined scoring systems to limit suffering.

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.