Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 26

Projects with a primary purpose of: Translational Applied Research - Human Endocrine and Metabolism Disorders

Project Titles and keywords

1. **CNS regulation of energy homeostasis**
    - Obesity, inflammation, food-intake, body-weight, glia

2. **Mitochondrial function in metabolic diseases**
    - Metabolism, heart, mitochondria, diabetes, obesit**y**

3. **Novel therapeutics for obesity and metabolic disorders**
    - Obesity, metabolic disorders, novel therapeutics

4. **The Rho-GTPase Signalling Network in Health and Disease**
    - Rac-GEFs, small GTPases, signalling, inflammation, metabolism

5. **Effects on metabolism and endocrine pharmacology**
    - Metabolism, Endocrine, Safety, Efficacy, Pharmacology

6. **Understanding metabolic phenotypes in mouse lines**
    - Obesity, insulin, diabetes, mouse, genetics

7. **Identifying diabetes, obesity and metabolic disease therapeutics**

8. **Models for Endocrine Neoplasia**
    - Pituitary, Parathyroid, Pancreatic Islets, MEN1, NETs

9. **Metabolic dysfunction and degeneration**
    - Obesity, diabetes, vascular disease, Alzheimer's

10. **Metabolism and Toxicity in Non-human Primates**
    - Pharmaceutical, Regulatory, Primate

11. **Cell Therapy for Diabetes**
    - Type 1 Diabetes, Regenerative Medicine, Islet Transplantation, Reprogramming, Transdifferentiation

12. **Mechanisms regulating obesity, growth and dementia**
    - Satiety, growth, Alzheimer's disease, dementia

13. **Regulation of glucose homeostasis in vivo**
    Diabetes, islets of Langerhans, insulin secretion, insulin action

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<tr><td>Project 1</td><td>CNS regulation of energy homeostasis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Obesity, inflammation, food-intake, body-weight, glia</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
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<tr><td>Basic research</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Dysregulation of energy homeostasis, the balance between food intake and energy expenditure, contributes to obesity. The brain controls body weight by regulating food intake and energy expenditure. Published studies using rodents have identified neuronal circuits in the brain, vital for the regulation of energy homeostasis. Although they make up a substantial proportion of the cellular mass in the brain (>50% in some areas), the contribution of non-neuronal cells, such as glia, to the regulation of energy homeostasis remains unclear. The objectives of the proposed work are as follows -

1. To determine how obesity and diets which promote obesity cause changes in inflammation and glial-cell signalling in the brain.
2. To use drugs and genetically modified animals to determine how these changes in inflammatory and glial-cell signalling in the brain promote obesity and associated disorders including changes in food intake, body weight, blood glucose control and memory.
3. To use this information to identify potential new</td></tr>
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	drug targets for the treatment of conditions characterised by altered energy balance such as obesity and its associated disorders e.g. 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)?	Obesity effects 25% of the population and is associated with complications such as diabetes, cardiovascular disease, dementia and cancer, which all significantly reduce quality of life and increase mortality. This project may benefit human health by providing new knowledge regarding the molecular mechanisms underlying obesity and its associated disorders. Furthermore, this project may also lead to the identification of new drug targets for the treatment of obesity and its associated complications.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will utilize up to 1160 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 know that obesity and high-fat diets cause inflammation and glial cell activation in the brain. However, what is unknown is why this happens and what the consequences are. We will use animal models to identify novel molecular pathways altered in the brain by obesity and high-fat diets. We will then use drugs (injected into mice) and genetic modification mice to examine what happens to food intake, body weight, blood glucose control and memory in mice when you manipulate the activity of these molecular pathways. This will inform us if/how these molecular changes contribute to the development of obesity, and whether they may be potential drug targets to reduce obesity and treat its complications. These studies are expected to fall under a moderate severity category. It is expected that these studies may result in the following adverse effects: stress; complications from obesity e.g. insulin resistance, increased thirst/drinking, increased urination; weight-loss; surgical complications e.g. swelling, blood-loss, transient minimised pain following surgery; complications of genetic modification which may include altered behaviour and developmental defects. At the end of the studies, the animals will be killed

	and tissues may be taken to advance the scientific aims of the project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because obesity is a complicated multifactorial disease encompassing many organs it is not possible to model the complex multifactorial nature of the disease in isolated cell lines. In particular the brain, which is the main organ that will be studied under this protocol, consists of multiple cell types whose functional interactions remain poorly understood. Other alternatives, such as computer simulations cannot be used until the unknowns have been further elucidated.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For each new experiment, we will initially undertake pilot studies and then use this information to carry out statistical power analyses to inform us of the minimum number of animals that would be required to achieve statistical significance. When applicable we will use genetically modified mice to target the activity of proteins in specific cell types in the brain, which will enable more precise targeting of cellular activity not possible using drug administration. This more precise methodology will reduce the amount of variables within our studies and allow us to reduce further the number of animals used. Strategies for breeding genetically altered animals will be carefully designed to minimize the amount of breeding necessary to generate the number of animals needed for experiments, thus reducing the number of animals used. Littermates will be used as controls to minimise further animal usage and also to control for the effects of the *in utero* environment.
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 mostly commonly used research model for the study of obesity and the regulation of food intake; therefore, the vast majority of the existing research literature comes from this model. As such, the mouse as model to study obesity and the regulation of food intake has already been extensively validated by the scientific community. Mice have a similar digestive tract and endocrine system to humans and readily become obese when presented with a high-calorie diet. Mice are one of the only mammalian species easily

	amenable to genetic modification and can be used for identification of critical molecular pathways important for the progression of diseases. Furthermore, the ability to create cell-type specific and drug-inducible genetic modifications in mice allows for further refinement and specificity in the targeting of molecular pathways *in vivo*. A number of genetic mouse models of obesity are already commercially available. To date numerous genetic modifications identified to cause obesity in mice have also been found to result in obesity in humans validating the utility of the mouse a model for human disease in this context. A good example of this is the melanocortin-4 receptor. First implicated in mediating energy homeostasis and obesity through mouse studies, alterations in the melanocortin-4 receptor gene were later found to be responsible for up to 5% of cases of severe early onset obesity in children. To minimise the welfare cost to all animals undergoing surgical procedures aseptic technique will be used and perioperative analgesia administered.

Project 2	**Mitochondrial function in metabolic diseases**	
Key Words (max. 5 words)	Metabolism, heart, mitochondria, diabetes, obesity	
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 project aims to elucidate the mechanisms of common metabolic diseases such as diabetes, obesity and heart failure. It also aims to investigate possible novel therapeutic strategies, including via dietary manipulation, that aim to improve tissue energy levels and therefore function of the tissue, organ and whole body.	
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 incidence of chronic metabolic disease in the developed world continues to increase, associated with the rise in obesity. With no cure currently available for many of these diseases, including type 2 diabetes and cardiovascular disease, it is vital that we develop a greater understanding of the biological mechanisms underlying these diseases and the high mortality rates associated with them. In so doing, we can understand why mortality associated with these conditions is currently so unacceptably high, as well as identifying possible targets of new therapies that could positively impact on patients' health, improving survival and quality of life. This study has the potential to benefit millions of people worldwide with these conditions by a) identifying novel therapeutic targets, and b) working with stakeholders, including health charities and industry to move our findings into public health advice	

	or therapeutics.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice. We would estimate a total of 2200 animals over the course of this work.
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 some animal models of diabetes and obesity, as well as animals exposed to low atmospheric oxygen conditions (such as a person might experience at high altitude) as low tissue oxygen levels are a key feature of many of these conditions (including obesity, diabetes and heart failure). These animals will have their diets altered (whilst continuing to provide adequate nutrition for normal health) and may be injected with drugs or other substances that could improve their condition. The mice may be provided with running wheels in their cages to test their capacity for exercise, but they will not be forced to run and as this exercise aspect is voluntary it can enrich their environments. We may also measure blood pressure in animals with a tail cuff. The majority of animals in this project will experience mild conditions, but in a small number of animals e.g. some models of disease they could experience a more moderate degree of discomfort. Generally expected outcomes in these animals include increased tiredness and lethargy. Some animals may lose appetite and thus body weight, but this would be expected to be temporary and will be closely monitored. At the end of procedures animals will be killed by a humane method and their bodily tissues collected to provide a significant amount of data for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex nature of metabolic disease requires an interplay between numerous tissues e.g. heart, liver, fat, skeletal muscle, and as such it is not possible to study such systemic conditions in cultured cells. Moreover, cultured cells do not exhibit the functional properties of tissues inside the body (e.g. cultured heart cells do not contract against blood pressures, as the heart muscle does). It is also not viable to collect samples of some internal organs (such as heart and liver) from human subjects (particularly healthy humans who would not be undertaking invasive operations), and so we must

	use animals in order to be able to collect such organs and to study the function of them.
2. Reduction Explain how you will assure the use of minimum numbers of animals	From each animal, multiple tissues will be collected and analysed to ensure that the best use is made out of every single animal used and total numbers therefore minimised. Wherever possible, we use cells or human subjects rather than animals, for instance we can take fat or muscle biopsies and blood samples from humans without causing any lasting harm, and despite their functional limitations we can use cultured cells to understand some genetic pathways.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats and mice are the least sentient animals which are appropriate for the study of mammalian metabolism. Established disease models (including genetically-modified animals) are available for the study of diabetes and obesity. The choice of models will be determined by a) those that best represent the human condition, b) those that allow us to address a particularly scientific question, and c) those that minimise distress/harm to the animal. Welfare costs will be minimised by careful selection of models used, as well as regular monitoring and scoring of individual animals with the application of early humane endpoints such that animals are humanely killed to avoid unnecessary suffering.

Project 3	**Novel therapeutics for obesity and metabolic disorders**	
Key Words (max. 5 words)	Obesity, metabolic disorders, novel therapeutics	
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
	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)	There is growing consensus amongst health care experts that the global epidemic of obesity will be one of the leading causes of mortality and morbidity for current and future generations. Obesity drives the development of a number of pathological conditions that are often comorbid (medical conditions present simultaneously in a patient) and have therefore been described by the term “metabolic syndrome”. The major components of metabolic syndrome are clyslipidaemia, atherosclerosis, high blood pressure and type 2 diabetes. All of the features of metabolic syndrome increase the risk of serious vascular events including heart attack and stroke. Drug therapy may be a suitable option for people who have been unsuccessful in losing weight with lifestyle changes. In Europe, only one medication, orlistat (Xenical), is approved for the treatment of obesity, the main driver of metabolic syndrome. However, the effect of orlistat on body weight is only modest, its use is associated with high rates of gastrointestinal side effects, and it may have negative effects on the kidneys. In the USA, lorcaserin (Belviq) and a combination of phentermine and topiramate (Qsymia) are also available for the treatment of obesity.	

	However, their effects are also only modest, and both may have negative effects on the heart. The overall aim of the project is to give support to the drug discovery phase of research projects for external clients. In particular, the objectives of the licence are:1 To validate pharmacological targets for treating obesity and/or metabolic disorders. Experiments will be carried out in rat and mouse disease models of obesity and metabolic disorders to test whether possible targets for drug discovery are appropriate. The results from these experiments will help decide whether a novel target is valid, and justifies starting a full drug discovery project. 2 To identify and screen compounds for treating obesity and/or metabolic disorders. Data generated in disease models will help decide whether to progress a compound into early stage drug development. 3 To test the possible side-effects of new agents that target indications other than obesity and/or metabolic for side-effect liability on obesity and/or metabolic disorder end-points. Data generated in disease models will show whether a potential new treatment for a disorder other than metabolic disorders affects metabolic disorder symptoms. Such data will help decide whether to progress the potential treatment into early stage drug development.
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 major potential benefit of this work is the discovery of novel drugs for the treatment of obesity and metabolic disorders, an area of unmet medical need. This project licence will enable drug discovery projects that are aiming to produce treatments which are superior, in terms of efficacy and side-effect liability, to current treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice will be used on this licence, as they are the lowest sentient species that can be used to assess the effect of novel agents on relevant metabolic parameters. It is expected that no more than 3000 rats and 3000 mice will be used over the 5-year life 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	Adverse effects (eg piloerection, mild sedation and salivation) are those associated with testing novel compound from early stage drug discovery projects, but the likelihood of occurrence is very low. Close monitoring and use of pilot studies, will help to keep

severity? What will happen to the animals at the end?	the incidence of adverse effects to a minimum. Another potential adverse effect is dehydration due to increased urine flow as a result of diabetes. To keep the incidence of this adverse effect to a minimum, additional water bottles will be provided. Although very rare, if a urinary tract infection occurs the animal will be killed humanely. Animals will be humanely killed at the end of all protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The in vivo models described in this licence application are employed to generate information about how the whole body responds once it has been given a compound. It is neither possible, nor ethical, to use human volunteers in early drug discovery. It is therefore necessary to use other whole body systems, animals, to find out how a living organism responds. The studies covered by this licence typically follow on from in vitro models/assays performed by our clients, The in vitro models provide useful information about which are the best chemical leads from a particular chemistry program to be select for further study. However, at present, in vitro methods cannot entirely predict and replace the in vivo models described by this licence, as the technology does not exist to simulate the complexity and diversity of the whole body system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals required per group and the experimental design are determined on the basis of power analysis, advice from statisticians, published data and previous results that have consistently identified target effects in a clear and unambiguous manner. Whenever possible repeated measure analyses will be employed to increase precision, maintain smaller group sizes, and reduce animal usage. For example behavioural measures from one subject might be recorded, and compared, both before and after drug administration, or on multiple occasions over time. Within each experiment a positive control is included to provide an internal control to compare the relative efficacy of the test compound and to assess the sensitivity/validity of the test procedure on a given test occasion. This good experimental design principle will

	avoid unnecessary replication of experiments.
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.	Purpose bred, adult free living animals of assured health and genetic status will be obtained from commercial suppliers, or from breeding.Animal suffering will be minimised by the following; • Conditions in the animal house follow current best practice, and items such as bone chews are placed in rodent cages for their stimulation. • Competent personnel will perform all studies on this project licence and adverse effects will be minimised by careful handling and the application of good technique. Guidelines on the limit of volumes of administration of substances and blood sampling will be strictly adhered to. Clear-cut end points are described in the possible adverse event description for the protocol covered by this licence.

Project 4	**The Rho-GTPase Signalling Network in Health and Disease**	
Key Words (max. 5 words)	Rac-GEFs, small GTPases, signalling, inflammation, metabolism	
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)	The main objective is to increase our understanding of the molecular and cellular processes that determine a healthy lifespan and that, when deregulated, can cause or worsen diseases such as chronic inflammation and type-2 diabetes. A better understanding of the biological mechanisms underlying human health and the causes of complex diseases will allow us to better treat such diseases in the future, by enabling us to use rational strategies in the development of more efficient therapeutics with fewer undesirable side-effects.	
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 expected benefit from this project is that we will gain a better understanding of the complex molecular mechanisms that underpin a healthy life-span and that - when deregulated - can cause or exacerbate complex diseases such as inflammatory and metabolic disorders. In the long term, the knowledge generated from this project might contribute to the development of more effective and less toxic drugs.	
What species and approximate numbers of animals do you expect to use	Mice. Approximately 2800 per year over 5 years.	

over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Over 85% of the mice will experience mild suffering at worst. The main adverse effect could be a mild and transient inflammatory response. Approximately 15% of the mice will experience moderate suffering at worst. The main adverse effects could be a more sustained inflammatory response or, in around 1% of the mice, health problems associated with weight gain during ageing caused by access to unlimited food supply. All animals will be killed by a quick and humane method at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use many different approaches to help us avoid the use of animals in research, including research on purified proteins or in cultured cell lines. However, some use of animals is necessary if we want to understand better the complex processes that allow us to live healthily and how they can be deregulated to cause or worsen diseases such as inflammatory and metabolic disorders.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will assure that we use the smallest number of animals that allows us to obtain meaningful results by employing good statistical methods and modern technologies that enable multiple measurements to be made and in the least invasive way.
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 best species to use for the objectives of this licence. Compared to humans, their physiological and disease processes are sufficiently similar to allow us to draw meaningful conclusions from our research. Furthermore, mice are very widely used in academic and pharmaceutical research, meaning that results obtained in this species can easily be compared to results from other research groups. Sophisticated methodologies have been optimised for use with mice to maximise the knowledge gained from the use of each animal. We only employ models that are widely accepted as reliable and were optimized to minimize harm and the numbers of animals used. We use non-invasive techniques where possible and attempt to keep up-to-date with new advances that offer further animal-welfare advantages. Our mouse research is done using an approach of limiting clinical signs, guided by animal technicians and vets, where any mice seen to be experiencing unexpected suffering are killed by a humane method.

Project 5	**Effects on metabolism and endocrine pharmacology**
Key Words	Metabolism, Endocrine, Safety, Efficacy, Pharmacology
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Compounds that interact with the endocrine system are known to produce profound effects in nature and humans even when the individual is exposed to very small doses. Any system in the body controlled by hormones can be detrimentally affected by hormone disruptors. Specifically, endocrine disruptors may be associated with the development of sexual development problems such as feminizing of males or masculinising effects on females, etc. It is therefore important, where possible to produce drugs and other chemicals that are well tolerated and as free as possible from such side effects. Much of the work conducted under this Licence will be concerned with side effect profiling with the ultimate aim of minimising side effects such as endocrine disruption.

This Licence also allows for efficacy testing that will, for example, assess potential useful drugs affecting metabolic processes and correcting effects that may occur when metabolic imbalances occur eg in diabetes, high cholesterol and anaemia. Such conditions/disease states often affect large numbers of people.

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)?

Governments require (and the public expects) that substances we are exposed to are safe or their hazards are well understood. It is an internationally mandated legal requirement. Regulatory approval is required to allow drugs to be tested in human or

veterinary trials, or for chemicals, agrochemicals to be marketed. Novel drugs may be developed with reduced or limited metabolic/endocrine side effects or a side effect profile that may be better tolerated than currently marketed products. Alternatively, novel drugs that produce beneficial effects via actions on the metabolic/endocrine system may also be assessed and developed as part of this licence.

What types and approximate numbers of animals do you expect to use and over what period of time?

The species and anticipated usage over the lifetime of the Licence (5 years) are below: Rat: 7,300 Mouse: 2,500 Guinea pig: 500

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Early studies are performed on the basis of limited information and there may be uncertainty regarding the severity of the response. Most animals are expected to experience no more than mild transient effects such as weight loss or changes in demeanour. A small percentage of animals may show more significant adverse effects indicating moderate severity eg. a very small number of animals may potentially experience severe adverse effects were it not for humane end-points (early intervention or humane euthanasia) to prevent unnecessary suffering. Animals in surgical studies may experience some adverse post-operative effects similar to those experienced by human patients, however, supportive treatments are given to eliminate or minimise these and appropriate humane endpoints are again applied. All surgical procedures are performed under anaesthesia, with pain relief and/or antibiotic cover provided during and after, as appropriate. On study completion, some animals may be re-used in other studies, but most animals are humanely killed using an appropriate method.

Application of the 3Rs

Replacement

Although non-animal (lab bench or computer based) studies can provide useful supporting data to limit and decrease the number animal studies, meaningful and reliable evaluation of whole body exposure can only be comprehensively achieved in studies using intact animals where all the organs and systems are intact, interacting with each other and interacting with the compound, yielding a naturally complex interdependent system.

For this reason, in vitro and ex-vivo test systems in isolation remain inadequate alone. Use of in-vivo animal models remains a mandatory legal requirement; currently, for many of the study types in this project, there is no scientifically, ethically or legally acceptable non-animal alternative available.

Reduction

A logical tiered/sequential approach is generally adopted. Information is reviewed to decide whether testing is appropriate and ethically acceptable and the studies in a program are designed to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals. The numbers of animals used are kept to the minimum commensurate with meeting study objectives and regulatory requirements and further input from statisticians used where appropriate, to ensure robustness and relevance of the scientific data produced.

Where study designs allow, common controls may be used whereby a number of test substances under investigation may be tested and where comparison against a control is required, a single control may be used against which all the test substance groups may be compared thereby reducing the total number of animals required for testing.

Refinement

This project uses rats, mice and rarely guinea pigs.

The animal models described in this Licence are considered to be the most refined as consideration has been given to the methods being the least invasive to the animal whilst maximising the likelihood of generating quality scientific data that will answer the requirements of the piece of work being conducted.

All animals are monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).

Study designs are reviewed and new methods considered as technology best practice and standards improve and advances become adopted and approved by international regulatory agencies.

Wherever possible, experimental samples are collected under anaesthesia or post mortem to minimise any potential suffering. In some circumstances safety markers will also be collected from the animals maximizing the data from individual studies. Maximising data decreases use of further animals and collecting samples post mortem or from terminally anaesthetised animals, minimises suffering.

The use of biomarkers has proliferated greatly over recent years and continues to do so and therefore many studies that used to be conducted using disease models have been refined. In such cases, assessment of biomarkers in the blood of animals has replaced the need for the animal to experience the full condition/disease.

Project 6	**Understanding metabolic phenotypes in mouse lines**
Key Words	Obesity, insulin, diabetes, mouse, genetics
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Metabolic disorders such as obesity and type 2 diabetes are a highly important set of health problems. At an individual level, people's quality of life and lifespan are affected as metabolic disorders also predispose to diseases such as heart disease, atherosclerosis and cancer. At the level of society, care and treatment of people with metabolic disorders are a significant drain on healthcare funding, as well as a major cause of loss of productivity.

We know that both genes and the environment are important in causing the majority of cases of metabolic disorders and we understand quite well the environmental factors involved, such as too little exercise and too much calorie-rich food. In contrast, it is suspected that there are a large number of undiscovered genes which, when defective, also increase the risk of metabolic disorders.

Using our knowledge of human diseases and genetics, we shall study these genes in mice by altering the mouse's version of the gene and then testing (called "phenotyping") the mouse to see whether it develops the disease. If it does, this is strong evidence that the gene is important for human disease.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

We need to have a better understanding of the biology of metabolism to produce better treatments. Although scientists have been working on metabolism for many years, our understanding is still limited and new ways in which the body controls

metabolism remains undiscovered. This project hopes to identify new regulators in metabolism, leading to further studies on the function of relevant genes. This project is the first step towards fully understanding what newly identified genes do. We hope that the genes uncovered by this project will be relevant to humans. This will facilitate the development of future research paths and new treatments, therapies and screening tests to help people properly understand and manage the metabolic aspects of their health.

What types and approximate numbers of animals do you expect to use and over what period of time?

Over the next 5 years, we will use up to 25000 mice (including genetically-modified) for breeding and up to 6500 mice for experimental procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The main adverse effect comes from the effect of high energy diets to mimic human metabolic conditions. The mice will increase weight and body fat, which over a long time in susceptible mice can lead to chronic health concerns like diabetes and cardiovascular problems. Our studies will be completed before mice develop these symptoms and so we expect a moderate level of severity for these studies. At the end of the studies, the mice will be killed using humane methods, with blood and tissues collected from them for measuring metabolic factors like cholesterol or fat levels in the liver.

Application of the 3Rs

Replacement

We shall only look at genes where there is good preliminary evidence that they are involved in metabolism before starting any work on mice. This means looking at other sources of information (human-based genetics, other published human and mouse work, computer databases or cell models) and deciding whether a particular gene is a candidate for study. If this information does not exist, it must be generated first before any animal work is considered.

Metabolism is a complex process, involving many different organs (e.g. fat, heart, brain, liver) and different types of signal (e.g. hormones, nervous, proteins). The current state of *in vitro* technologies cannot adequately reproduce the complexity needed for the intended studies.

Reduction

We intend to use mouse colonies that already exist, using national and international repositories rather than create them ourselves in this project. By avoiding duplication, we significantly reduce the number of animals we use. The work is also carried out in a facility with strict environmental controls, so we reduce the variation in our mice and require fewer to look for metabolic changes.

For each experiment, we intend to use several small batches of mice, rather than one large set of animals. This means fewer mice are used for breeding and produces

fewer unrequired offspring. It also increases the chances of generating solid, reproducible data by using advanced statistical tools that are designed for many batches of mice.

Refinement

Mice are a suitable species for this project because they have similar organ systems to humans which have similar metabolic functions. Mice also have very similar genomes to humans, so the majority of human genes we would like to study will exist in mouse.

Many mouse strains can develop the symptoms of metabolic disorders found in humans, including obesity and elevated insulin levels. This can often be done simply by giving the mice free access to an energy-rich diet and the effect can be easily assessed by weighing the mice. We can also perform investigations on mice that are similar to those performed on people with metabolic problems, such as measurement of insulin and cholesterol in blood samples or by measuring body fat using by use of X-rays (known as DEXA scans). As a commitment to the 3Rs, we intend to use experimental variations which minimise the stress the mouse is subjected to, for example when shorter fasting times can be used instead of longer fasts. These methods are well established both in humans and in mice, and provide a way of being able to compare results between species.

Mice are social animals, so in this project they are always group housed, except for specific periods where single housing is required for scientific reasons (such as being able to accurately measure food intake from a single mouse). The mice are provided with environmental enrichment such as cardboard tunnels and nesting materials to facilitate normal behaviours. We use a sophisticated database system for tracking the tests the mice have had and for monitoring of health and welfare concerns. This allows live reporting on the condition of every mouse, so that if a mouse is found to have a health problem, swift decisions can be made.

Project 7	**Identifying diabetes, obesity and metabolic disease therapeutics**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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 first WHO *Global report on diabetes*) demonstrates that the number of adults living with diabetes has almost quadrupled since 1980 to 422 million adults. This dramatic rise is largely due to the rise in type 2 diabetes and factors driving it include overweight and obesity. Current strategies for treating this problem revolve around drugs with a single mode of action. It has become clear that these medicines are not adequate for treating type II diabetes and its underlying cause – fat-induced resistance to insulin action (insulin resistance). This project aims to identify multiple molecular targets for the treatment of insulin resistance and develop more effective drug treatments.	
What are the potential benefits likely to derive from this project (how science could be	This project will identify which of the molecular mechanisms involved in the regulation of energy balance, body fat content, blood lipid levels, and	

advanced or humans or animals could benefit from the project)?	adipose tissue inflammation that are suitable targets for combination therapies of insulin resistance, and the consequent effects on glucose homeostasis. Drug treatments will be assessed with the expected conclusion to at least one potential therapy – the use of Free Fatty Acid Receptors agonists.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse. 5000 over the 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?	Mice will be bred and made insulin resistant either via a high fat diet or by genetic modification. They may also be given high fat diets to induce a mild degree of obesity, i.e. physiological parameters will be measurably altered, but not to the extent that cause disease (in this case diabetes) and the discomfort experienced will be transient. The main interventions that will cause distress or pain to the mice are administration of treatment compounds, repeat blood sampling and dosing. Blood sampling is done by nicking the tail and massaging the tail to get the droplets of blood. This causes very little pain which will be controlled by the use of local anaesthetic. When repeat dosing by injection, the site of administration will be rotated so that each animal is fully recovered before the next dose. Implantation of a minipump requires general anaesthesia with the resulting stress and disorientation upon recovery. This technique takes only a few minutes per mouse and pain will be controlled by post-operative analgesics. All other procedures are non-invasive.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Obesity-related diabetes involves interactions between many different organs of the body including the brain and nerves, muscle, fat, liver, intestines, pancreas, and the immune system. The only way to investigate diabetes fully is to use animals.
2. Reduction	Statistical analysis of the range of responses to

Explain how you will assure the use of minimum numbers of animals	procedures will be utilized to give the number of mice needed to ensure a successful result is achieved, and this number and no more will be used in the studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are considered to be the lowest vertebrate animal that displays the same obesity-induced insulin resistance as humans. Anaesthesia will be used whenever an invasive technique is used on an animal. These techniques will be kept to a minimum required for the study. Where possible, treatments will be given in food or water rather than by injection, and studies will be kept as short as possible.

<table>
<tr><td>Project 8</td><td colspan="2">Models for Endocrine Neoplasia</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Pituitary, Parathyroid, Pancreatic Islets, MEN1, NETs</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>x</td><td>Basic research</td></tr>
<tr><td>x</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td>X</td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">Summary

This project uses mouse models of endocrine cancers to investigate potential causes of tumours and treatments.

Brief Scientific Background

Endocrine glands produce and release chemicals, called hormones, which have effects on other organs. For example, excessive production of a hormone by parathyroid tumours, located in the neck, causes bone thinning (osteoporosis), with too much calcium in the blood and urine, which causes kidney stones.

Endocrine tumours, which have an incidence equivalent to that of prostate and colorectal cancers, may be inherited, due to the presence of an abnormal (mutated) gene, which is passed by parents to their children. Current treatments with medicines and surgery are not satisfactory and the study of mouse models will allow the causes to be defined and new</td></tr>
</table>

	treatments to be assessed. **Outline of the project plan** We have established mouse models for inherited endocrine tumours, which are representative of the disease in man, and propose to: characterise the molecular mechanisms of tumour formation; develop and test novel therapies which may be used in man; and try to make a cell line, which could be used in some experiments to replace animals. We also aim to find the normal function of the gene mutated in the endocrine tumour which may be helping the embryo to develop; to determine this we have to study embryos, if mice with abnormalities in both copies of a gene are not born and die in the mother's uterus (womb).
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 should lead to a better understanding of biological mechanisms involved in endocrine tumour formation, and help to develop novel therapies such as: gene replacement therapy to replace the faulty gene in the tumour cells with a normal copy of the gene and thus restore function; epigenetic drugs that remove potentially harmful chemical modifications to the DNA, and tumour-binding peptides that may reduce the potential of the tumour cells to grow, which may have a clinical impact on the management and prevention of endocrine tumours, which result in significant morbidity in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We propose to use mouse models, as mice provide rapid and efficient breeding while maintaining a high degree of similarity to humans. Almost all of the genes expressed by humans are expressed in mouse, with research by the National Human Genome Research Institute showing that only 10 of 4000 genes studied were found in one species and not the other. Furthermore the regions of DNA coding for genes show >85% similarity between humans and mice, therefore any genes and pathways found to be causative of endocrine neoplasia in mice, are likely causative in humans. While several multiple endocrine neoplasia type 1 (MEN1) models have been generated our conventional MEN1

	knockout model is the only one to demonstrate hypercalcaemia which is found in 95% of MEN1 patients and thus is likely to represent a closer similarity to MEN1 than the other models. Over the five year duration of this licence we expect to use no more than 20,000 mice.
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 application is expected to be moderate. The adverse effects experienced by any mouse will be monitored carefully and reduced wherever possible. The mice, will experience no harms until tumours develop when they will be constantly monitored for adverse effects. The mice will be examined frequently for well-being, weight loss and other symptoms, and if they appear to be suffering they will be humanely killed. The expected adverse effects of mice with endocrine tumours include symptoms similar to that of human patients, and in the worst case lead to symptoms associated with high or low levels in the blood of calcium and glucose respectively, whilst there may also be effects of old age. There may also be side effects due to the drugs given and these include weight loss and dehydration. There may also be adverse effects occurring due to the tests we are performing e.g. blood sampling and housing mice singly in metabolic cages for up to 5 days, after which male mice may not return to social housing. We will take steps wherever possible to decrease these adverse effects and if welfare is compromised in any mouse it will be killed immediately in a humane way. These steps include careful monitoring of mice for visible signs of distress, housing mice individually for the shortest possible timeframe possible to get scientifically meaningful results, and exposing singly housed males to soiled bedding to maintain exposure to the scent of previous cagemates and thereby prevent isolation and aggression. In addition pain relief will be administered to mice, for example if repeat blood sampling or anaesthetic procedures are performed, as this will reduce discomfort for the mouse. If there is no compromise to welfare then mice will be killed in a humane way at the end of the

	protocol they are enrolled in.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Endocrine glands produce and release chemicals, called hormones, which target other organs to maintain the physiological balance of the whole organism. It is not possible to fully replicate this dynamic system without the use of live animals. This investigation, which is over a long period of time and involves multiple organs, cannot be done using cells or isolated organs. The disease develops in older animals, and there are many complex influences on its development, which can only occur in the living animal. Assessing the effects of new medicines cannot be undertaken in humans because their effectiveness and side effects are unknown. There is therefore no alternative to using animals for these studies. We do, however, strive to develop models that can reduce the number of mice needed, for example by trying to grow cells taken from mouse endocrine tumours in the laboratory. We also continually communicate with experts in the field to keep up to date with possible alternatives to the use of live mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The estimated number of mice required for our studies is based on our previous experiences, and mathematical calculations estimating the numbers required to obtain meaningful results. The number of mice required for any particular study vary considerably, depending on: the variability of the measurement; individual variability amongst genetically altered mice; variability between mouse strains; gender differences; variability due to age; environment; and the effectiveness of the intervention (e.g. therapy). Wherever possible we aim to standardise a number of these variables e.g. age/gender/environment to reduce variability and ultimately reduce the number of mice required. Furthermore, we preserve mouse lines where possible, by freezing embryos, thereby negating the need for continuous breeding.
3. Refinement Explain the choice of species	Only mice will be used in our studies and were chosen as they represent the lowest mammal, displaying a sufficiently similar endocrine system,

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.	and genetic similarity to humans. We have previously established and characterised mouse models for use in this study and further models of endocrine tumours based on human diseases will be established using our prior experience with techniques and facilities that we have readily available. Our conditional Men1 models are particularly useful for assessing the effects of drugs since unlike the conventional Men1 KO models, all mice develop pancreatic tumours, and all tumours develop at the same age, allowing for more accurate comparisons between treatment groups. Thus, the age of tumour development is more defined and the organs in which they will develop; thereby making it easier to predict when the mice may begin to have symptoms that could cause distress. Furthermore tumours can develop as early as 5 months, therefore reducing the length of the protocol. We are particularly keen to minimise severity and increase the welfare of these animals. To ensure this we will use observational methods and will refine and adjust these methods in the light of experience gained during the course of this work. In addition, we are constantly seeking to refine our protocols and techniques by searching emerging literature and seeking advice from experts in the field. This includes the use of pain relief and using the least invasive methods for delivery of drugs and molecular therapies

Project 9	**Metabolic dysfunction and degeneration**	
Key Words (max. 5 words)	Obesity, diabetes, vascular disease, Alzheimer's	
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)	Obesity worldwide has increased dramatically and is a major factor for escalating rates of diabetes, atherosclerosis and nonalcoholic fatty liver disease (NASH), all of which increase the risk of Alzheimer's disease (AD). This places an enormous burden on our health systems and society generally, which is likely to worsen over the next 10-20 years if effective actions are not taken soon. These diseases are strongly linked with reduced sensitivity to two hormones, leptin and insulin. Leptin plays a key role in the maintenance of body weight by acting in a brain area called the hypothalamus to reduce food intake and increase energy expenditure. However, its effects are diminished in obese individuals, despite increased levels of leptin in the blood. This is known as leptin resistance and occurs in the hypothalamus. At present the molecular basis of leptin resistance is unclear. The hormone insulin is important in regulating sugar levels in the blood. Excess fat, which is considered the primary risk factor for of Type 2 diabetes, is linked closely to reduced sensitivity of muscle, liver and fat to	

	insulin. This is termed insulin resistance and also occurs in atherosclerosis and fatty liver disease. Leptin and insulin resistance are linked by chronic inflammation. There are few effective drug treatments for obesity and none that overcome hypothalamic leptin resistance. Currently, only two medicines, metformin and pioglitazone, improve insulin resistance in diabetes patients, thus new drugs that help to reduce insulin resistance and improve control of blood glucose in obese diabetic patients would be welcome. The enzyme BACEI cuts a protein called amyloid precursor protein (APP) into smaller fragments, some of which are considered toxic and responsible for AD. Diabetes, obesity and AD have features in common, reduced function of leptin and insulin and reduced metabolism of glucose. We find that high fat intake and blood glucose raises BACEI activity with increased generation of amyloid peptides (fragments from APP). By reducing the activity levels of the BACE1 enzyme genetically or by using drugs we have demonstrated improved hypothalamic leptin sensitivity (less obesity) with increased insulin sensitivity and glucose control (less diabetes) and improved cardiovascular function. Our hypothesis is that raised Bacel activity generates multiple peptide fragments that modify metabolism and hormone function. We now wish to extend this work and define the mechanisms underlying these outcomes at the level of the cell. If our hypothesis is validated by this work, BACEI inhibitors (currently in clinical trials for AD) could be re-purposed to investigate their potential for treatment of obesity-driven metabolic disease.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We do not understand how the brain monitors body fat and glucose levels, although we know that neurons in the hypothalamus can influence blood levels of hormones and metabolites and have a major impact on long term body weight and short-term regulation of blood glucose. High fat diet and excess glucose alter brain peripheral organ (e.g muscle, liver and adipose) function, giving rise to obesity, diabetes and atherosclerosis. If we can demonstrate that

	inappropriate high levels of Bacel activity and the peptides that it releases are directly correlated with these chronic disease states, we may be able to develop novel treatments to reverse or prevent these conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	The main species studied will be mouse. A small number of rats will also be used. The maximum total number of animals used in experimental procedures over the duration of the project will be 2100, with another approximately 4000 being used for the necessary breeding of informative lines of genetically altered animals.
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 usually be fed altered diets to promote obesity, diabetes and icr atherosclerosis. These diets should not cause overt adverse effects. They may receive drugs in water or the diet or by injection. The drugs are not expected to have any toxic side effects. Occasionally animals have to be given the drugs over a period of time and if this can't be done in food or water it is often better for the animal to have an implant that releases the drug over time. Because we need to target the hypothalamus in the brain, sometimes to get the drugs to act in the way we need animals need to have small injections of drug into or near this area of the brain. If the drug is to be given over a period of time a very fine tube (cannula) will be implanted. If we need to put in an implant or cannula this is done under anaesthetic and animals are given painkillers to minimise the pain when they wake up. Some animals will be given a drug that will make them diabetic. Occasionally animals may be implanted with a device that can measure body temperature and/or blood pressure (telemetry devices) The main adverse effects expected from these procedures relate to the implant surgery or the development of overt diabetes. The appearance of diabetes will be monitored closely by regular checking of blood glucose levels with animals given glucose (if episodes of low blood glucose) or treated with insulin (to prevent very high levels of blood glucose. When animals have completed the in vivo studies, they

	are humanely euthanased to prevent any suffering and so that we can further qain information from them by studying their tissues.
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 an animal regulates its body weight, glucose levels and energy expenditure are complex, involving the interplay of central (brain) and peripheral organs. These cannot easily be mimicked in vitro. Although important in advancing our understanding of the actions of hormones and nutrients on signalling and metabolic pathways use of cell lines do not faithfully mimic all aspects of the behaviour and physiological/pathophysiological interactions of these systems in the intact animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding will be optimised to produce the genotype(s) required, with the programme subject to regular review to optimise numbers to experimental demand. The numbers bred will be managed carefully to avoid over-supply. The number of animals will be minimised by careful experimental design and appropriate statistical analysis. By using appropriate in-vitro systems throughout we will reduce animal usage significantly. We will determine the minimum number of animals to achieve significance for each scientific end point required, with continuous recognition for each protocol to allow, wherever possible, multiple experimental outcomes from a single animal. At the end of each protocol, mice are humanely killed and tissues harvested, frozen, archived and stored at -80. We share tissues and data with others where possible. Unnecessary production of GAA will be avoided by searching resource databases (e.g. Jackson Laboratory, EMMA, IMSR, MRC Harwell, EUCOMM)
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	The mouse is the best alternative species currently available for (I) ease and reproducibility of genetic manipulation (ii) analysis of metabolism and metabolic pathways, which mirror humans (iii) the majority of AD models are mouse, mostly transgenic. Rats may be used although less frequently. Their larger size make them a more suitable model for some surgical interventions and/or for assurance of cross-species

take to minimise welfare costs (harms) to the animals.	effectiveness of drug intervention. Use of high fat or high fat with cholesterol or fructose diets are least harmful interventions and are standard methods to induce obesity, atherosclerosis, and increased risk of diabetes, AD and NASH in mice and rats. These mimic human westernized diets. As we are investigating diseases linked strongly with aging, we need a species that mirrors the human condition(s) as close as possible whilst maintaining a lifespan that is short enough for age-related changes to be examined within the duration of the funding. We have highly trained and well experienced staff to conduct these studies. All new staff are rigorously trained. All studies are carried out under the guidance of the local veterinary team.

Project 10	Metabolism and Toxicity in Non-human Primates
Key Words	Pharmaceutical, Regulatory, Primate
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

(b) translational or applied research with one of the following aims:

Yes (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

This project enables the programme of regulatory metabolism, immunology and toxicology studies in the non-human primate (macaques and marmosets), and the validation or investigative studies which enable the regulatory programme.

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)?

New medicines have the potential to benefit in new or improved disease treatments. Before potential new medicines are administered to humans their safety must be evaluated. This testing is a mandatory legal requirement and provides information on risks to people taking new medicines. At present there are no alternatives that don’t use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment.

What types and approximate numbers of animals do you expect to use and over what period of time?

Macaques (cynomolgus and rhesus) 7000 Marmosets 500 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 levels of severity? What will happen to the animals at the end?

Procedures carried out during these studies include: : Dosing (eg by oral administration, injection, infusion, insufflation), : Blood sampling or collection of urine for measurement of different components as changes in these may serve as early indicators of toxicity. Doctors for similar reasons often take blood and urine samples

from humans. : ECG monitoring to assess changes in heart function (e.g. number of heart beats per minute). This technique is also used by doctors to assess heart function in humans. : Examination of the eyes using a similar device to that used by opticians : Examination of more unusual parameters, eg retinography, placement of subcutaneous vascular access port or tissue biopsy under general anaesthesia, seminology (sampling by direct stimulation), body temperature by rectal thermometer (such as a doctor might use for a small child). A degree of restraint or confinement may be required for some procedures. The animals are trained using positive reinforcement (treat rewards) to move about the cages for handling/procedures, and to sit in restraint chairs. Some animals are re-used, but most animals are humanely killed at the end of the study by an overdose of anaesthetic to allow detailed examination of the organs. The majority of animals are expected to have mild adverse effects such as slight weight loss. A small percentage of animals may show more significant adverse effects e.g. more marked weight loss, or changes in appearance or behaviour (e.g. reduced activity) indicative of moderate severity. Humane end-points are applied, under veterinary guidance as necessary.

Application of the 3Rs

Replacement

Pharmaceutical testing is a mandatory legal requirement and provides information on risks to people taking new medicines. At present there are no alternatives that don't use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment.

In vitro and in silico methods are used in combination with animal studies to inform study designs and assist in understanding of potential toxicity but cannot yet replace in vivo studies.

We maintain a constant awareness of regulatory guidance and ensure that where non-invasive methods exist which fulfil the regulatory requirement they are used in preference to animal studies.

Reduction

The numbers of animals used in any particular study are generally linked directly to those indicated in the published regulatory guidelines. Animal numbers are kept to the minimum commensurate with meeting the objective of each study.

Refinement

We use non-human primates when other species (dogs and/or pigs) are unsuitable by one or more of the following criteria:

: kinetic or metabolic differences from man;

: species specific pharmacological or toxicological response,

Or when only primates are suitable by the following criteria:

: relevant toxicity or pharmacology which is only shown in a primate

: study design requires assessment of effects on organ systems or receptors for which primates are the only relevant model.

All procedures are subject to ongoing assessment and technique improvement and we participate in cross-company working parties on best practice. Animals are regularly reviewed for general health and veterinary staff are on call at all times to assess and ameliorate adverse events.

Project 11	Cell Therapy for Diabetes
Key Words	Type 1 Diabetes, Regenerative Medicine, Islet Transplantation, Reprogramming, Transdifferentiation
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The purpose of this project is to generate an alternative supply of islets for transplantation for the treatment of diabetes. This will be achieved by reprogramming the exocrine part of the pancreas that is normally left over at the end of the islet purification procedure. The reprogrammed cells will be characterised in vitro for insulin content and ability to secrete insulin in response to graded changes in glucose concentration in the culture media. However, on occasions it will important to characterise how the cells behave in a diabetic animal. We propose to use an immunodeficient mouse model so that the human cells will not be rejected. The mouse will be rendered diabetic following treatment with the drug streptozotocin. Following engraftment of the reprogrammed cells we will test for the presence of human insulin (C-peptide)in the blood of the mice as well as for blood glucose.

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)?

There are around 300,000 people with type 1 diabetes (T1D) in the UK. They are all dependent on exogenous insulin injections. However, it is very difficult, if not impossible to completely normalise blood glucose levels in this way. Many people with T1D suffer daily large excursions in blood glucose; either large increases (hyperglycaemia) or decreases (hypoglcaemia). Both have a huge impact on their well-being and an associated burden on the National Health Service. The only tested method that can normalise blood glucose levels without these excursions is islet transplantation. We have developed a protocol whereby the pancreatic tissue left

over from the islet purification procedure is efficiently reprogrammed into functional islets. We calculate that one pancreas could generate in the region of 10-20 transplantable islet units. This would therefore increase the number of transplants taking place in Scotland form around 20 per year to over 200. This would have a huge impact on the well-being of people with T1D, and in particular the substantial number who are seriously ill through the consequences of persistent hypoglycaemia.

What types and approximate numbers of animals do you expect to use and over what period of time?

We propose to use mice as a model to study whether our reprogrammed cells function in a manner similar to purified human islets in an in vivo environment. We expect to use up to 500 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 levels of severity? What will happen to the animals at the end?

We will purchase mice that have been genetically modified to disrupt their immune system. These mice will not reject transplanted human cells. We propose to render the mice diabetic by injecting them with a drug that destroys their islets. The resultant diabetic mice have elevated blood glucose levels but other than that are healthy with a normal life-span. In a typical experiment some mice will undergo surgical procedures, including implantation of cells beneath the kidney capsule, injection of cells into the hepatic vein, injection of cells subcutaneously or intraperitoneally, and kidney removal. At regular intervals blood samples will be taken to determine whether the grafted cells can normalise the elevated blood glucose levels. At the end of the experiment the mice will be humanely killed. The severity of the protocol is moderate.

Application of the 3Rs

Replacement

Before we can take this novel cell therapy for diabetes into the clinic we need to show that the cells work in a living environment. The mouse is the most appropriate animal to use since there are a number of immunodeficient strains available and the streptozotocin diabetic mouse is well–researched model. About 90% of the work undertaken in this project will be done in tissue culture dishes. However, as we make major advances in developing the protocol we need to ensure that the resultant cells also function in animals.

Reduction

The end-points are well characterised and this will allow us to use the minimum number of animals per group. The minimum number has been calculated on the basis of sound statistical principles, i.e. the minimum number of mice required to show normalisation of blood glucose levels and circulating human insulin when

transplanted with reprogrammed cells. The figure of 500 arises on the basis that 100 mice will be used per year: 5 mice per group, 4 groups per experiment and 5 experiments per year.

Refinement

The diabetic mice will be closely observed with regular recording of body weight; usually around the same time each day. Although healthy in many respects the streptozotocin-treated mice exhibit increased urination. Consequently the mice are given additional dry bedding and this is replaced regularly.

Surgery will be carried out be carried out aseptically by experienced personnel. The animals will receive appropriate analgesia/anaesthesia and peri-operative care as advised by the veterinary surgeon.

Project 12	Mechanisms regulating obesity, growth and dementia
Key Words	Satiety, growth, Alzheimer's disease, dementia
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The overall aim is to gain a fuller understanding of mechanisms regulating energy balance, growth and processes contributing towards the metabolic syndrome, its co-morbidities and Alzheimer's Disease (AD). Metabolic syndrome continues to rise across 200 countries recently assessed, and Alzheimer's disease International highlights the immediate need for basic and translational research, as they estimate 131.5 million cases by 2050 worldwide. We will investigate nutrient sensing mechanisms involved in satiety signalling and the processes involved in body weight regulation including gut derived hormone signalling, gut cell changes such as modification of the cell DNA in response to specific diet components, and how these changes affect signalling to, and in the brain. Emerging evidence in scientific literature shows that specific diet components e.g. types of fats, can cause inflammation-induced changes, leading to an imbalance in neuron numbers that drive overeating, compared to those that inhibit feeding. This project will examine the mechanism driving these changes, as well as how nutrients can impact on the development and progression of AD. We are seeking to understand if and how nutrients, and particularly those that affect levels of satiety hormones, can impact on this disease, as post mortem AD patient brains have been found to have altered levels of these hormones compared to normal brains. A number of nutrients such as fatty acids, vitamins and modified food ingredients are being recognised as risk factors for AD, thus we aim to determine their roles in affecting molecular changes in the brain that impact on cognition and dementia.

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 research should yield important new insights into components of the energy balance system and may identify novel targets for therapeutic intervention, or contribute to the design of successful dietary intervention strategies to tackle metabolic syndrome and reduce the effects of its associated co-morbidities. Elucidation of the mechanisms driving diet-to-gut to brain signalling is an important step toward the understanding of how and why the systems fail to restrain over-consumption. We expect to provide evidence based information to take forward into human trials, which will ultimately inform government policy advisers. Potential benefits for AD patients will be the identification of markers of early disease onset. We anticipate the work should generate firm evidence implicating specific nutrition-related risk factors in influencing disease development or progression. The results, promoted through peer-reviewed publication, will be of interest to the growing community of researchers worldwide who are addressing metabolic syndrome and AD.

What types and approximate numbers of animals do you expect to use and over what period of time?

Numbers will not exceed 2500 rats, 3000 mice and 400 hamsters over the 5 year 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 levels of severity? What will happen to the animals at the end?

Most experiments will involve diet manipulations to investigate physiological mechanisms, whilst some will involve surgery to explore the role of neuron changes and plasticity in growth and body weight regulation, which are only possible using live animals. Potential adverse effects include a small risk of loosening of cannula post-surgery, which will be minimised by good fixation technique. If it occurs, the animal will be immediately killed. Wound infections are a slight risk that can be minimised by good sterile surgical technique and subsequently maintaining appropriate cleanliness of the area. Blockage of cannulas may occur (moderate risk), and attempts will be made to unblock these and if required animals will be anaesthetised. If cannulas cannot be unblocked, animals will be humanely killed. Other potentially harmful methods include glucose tolerance testing, taking blood samples and housing on grid floors. Animals showing adverse effects will be treated where appropriate or humanely killed. The proposed severity classification of the procedures listed in Section E are based on the worst case scenario. We anticipate that <25% of animals will be of moderate severity at worst. Animals will be killed at the end of the experiments and tissues analysed.

Application of the 3Rs

Replacement

The objectives are to identify and characterise known and novel molecular mechanisms of energy balance and body weight regulation and processes affecting AD development and progression. Where possible we will use in vitro cell line work, for example to test modifications, caused by dietary components, to cell DNA. As the ultimate aim is to translate these findings for human benefits, other than the cell line work, there is no alternative to the use of live animals as a source of tissue for analysis following physiological, nutritional, endocrine or pharmacological manipulation. These manipulations can only be performed *in vivo*.

Reduction

In general, studies will be designed to compare control with treatment groups but many studies will also have a longitudinal component (e.g. time in photoperiod), where individuals act as their own control (repeated measures analysis), thereby reducing numbers. We have a growing database describing gene expression variability between animals of the target species/strains which may enable power calculations to optimise group sizes, and we will consult with appropriate statisticians. Where possible we will use cell lines to characterise responses and also explants which allow multiple experiments from far fewer animals than *in vivo* manipulations.

The numbers of animals required are estimates, based on the scope of the project and the data to be generated; previous experience; the anticipated size of my group; the number and genotypic requirements of genetically altered lines and is dependent on funding and other variable factors.

Refinement

Siberian hamsters will be used because they have a system for adapting adiposity which humans are also likely to express. F344 rats are appropriate for photoperiod studies as genetic information and tools (e.g. microarray) are available and there are no alternatives to specific mutant mice used. In general, investigations will typically begin with *in vitro,* non-invasive or minimally invasive work such as cell line work, photoperiod or terminal studies undertaken first before moving on to more invasive procedures**.** Surgical procedures are performed under aseptic conditions, a refinement to reduce infections. Surgically prepared animals are group housed whenever possible as this appears to improve recovery. We have also established that daily handling of animals minimises their stress and allows injections whilst mildly restrained. Body composition scanning provides repeated measures data which reduces animal numbers considerably.

Project 13	Regulation of glucose homeostasis in vivo
Key Words	Diabetes, islets of Langerhans, insulin secretion, insulin action
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Diabetes is a multisystem disease, involving insulin secretion deficit in response to elevated blood glucose, and insulin resistance in multiple tissues, including the liver, the adipose tissue, the brain, the placenta (for gestational diabetes), and the enteroendocrine cells of the intestines. Also diabetes complications cause stroke and heart attacks, kidney failure, neuropathy and limb amputation and blindness due to retinopathy. More specifically this project aims to understand the molecular mechanisms behind pancreatic beta cell failure during the progression of obesity and insulin resistance in order to provide invaluable data to healthcare professionals and pharmaceutical companies to develop new tools and drugs to limit the devastating effects of this disease. Another objective of this project is to implement, develop and validate the technique of islets transplantation in the anterior chamber of the eye for serial imaging which will provide an instrumental tool for following the damages caused to the islets by diabetogenic insults and the potential recovery induced by new drugs and treatments.

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)?

Diabetes is one of the most challenging socio-health emergencies of the third millennium. About 350 million people worldwide are estimated to be diabetic (50% of whom are undiagnosed), but this number is rapidly increasing due to aging populations and sedentary lifestyles, with the prospective of exceeding 500 million cases in 2030. Every year, 1.5 million deaths can be directly attributed to diabetes. In Western countries, the economic cost of diabetes can exceed 15% of the budget of national health systems. Therefore, impact of innovative methodologies and technologies for diabetes management can be extremely high. This basic research is trying to identify molecular signalling pathways involved in protecting the human body against the devastating effects of over-nutrition, physical inactivity and obesity, contributing to the development of type 2 diabetes and its crippling complications. The immediate beneficiaries will be academics working in the field of cell biology, but this research has the potential to interest pharmacological drug companies and to translate into clinical research by identifying potential drug targets to protect the pancreatic beta cell and the liver against glucolipotoxicity. The patients suffering from diabetes are ultimately going to benefit from this research.

What types and approximate numbers of animals do you expect to use and over what period of time?

The licence allows for 10000 mice and 5000 rats over a period of 5 years, spread over 6 research groups.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Animals will be rendered diabetic either by genetic modification, administration of drug or a high fat Westernised diet. The severity of diabetes will be investigated by serial blood tests and glucose (sugar) administration either orally or by injection. It can also be necessary to perform some surgery on selected animals. All the procedures in this licence are classified as either mild or moderate and are done under local, general or terminal anaesthesia where appropriate to minimise stress and suffering of the animals. All the animals will be humanely killed at the end of the procedures. We will always consult with the NACWO NVS and CBS technicians to make sure we are up to date in applying the most refined methods.

Application of the 3Rs

Replacement

The maintenance of normal blood glucose requires the interplay between hormonal secretion from the islets of Langerhans and hormonal action on peripheral tissues such as the liver, the skeletal muscle and adipose tissues. Neuronal and endocrine

outputs from the gut in response to changes in hormonal signalling and nutrient availability also modify the net effect on blood glucose. Such complex interrelations cannot be reproduced *in vitro* and require a whole living organism.

Reduction

Animal usage is based on careful power calculations, performed with G*Power. For example, for glucose tolerance tests, we may need a total requirement of 22 animals (11 per genotype) per experiment. This requirement is based on a typical standard deviation in the measurement of blood glucose of ~4 mmol/L. So to detect abnormal glucose tolerance (20% difference compared to a normal 30 min peak of ~ 26 mmol/L during glucose tolerance tests in 12-week old WT C57BL/6 mice on high fat diet in our hands), the effect size d = 1.3, requiring a group of 11 mice per group to detect a change at a significance level (alpha) of 0.05 with 80 % power. [Campbell,M.J., Julious,S.A., & Altman,D.G. Estimating sample sizes for binary, ordered categorical, and continuous outcomes in two group comparisons. *BMJ* **311**, 1145-1148 (1995)]

Refinement

Mice are the lowest vertebrates in which genetic manipulation can be successfully achieved and where diabetes studies are well documented. Rats give a better yield of blood and tissues per animal than mice and could be preferred if the relevant strain is available.

Both species are well acclimated to live in cages and laboratory conditions.

All the procedures in this application are classified as either mild or moderate and are done under local, general or terminal anaesthesia where appropriate to minimise stress and suffering of the animals.