Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 13

Projects with a primary purpose of: Translational and applied research - Human Endocrine and Metabolism Disorders

Project Titles and keywords

1. **Models for parathyroid development disorders**
    - Parathyroid, parathyroid hormone, calcium, development

2. **Ghrelin and the control of insulin secretion**
    - Ghrelin, insulin, diabetes

3. **Transporters/receptors and tissue metabolism**
    - Insulin, muscle, metabolism, endocannabinoid, amino acid

4. **Proteolytic cleavage of the LDL receptor**
    - Cholesterol, Low density lipoprotein receptor (LDLR), protease, inhibitor

5. **Bone marrow adipose tissue as an endocrine organ**
    - Bone marrow, adipose tissue, calorie restriction, obesity, metabolic health

6. **In vivo profiling of novel drugs**
    - Pharmacokinetics, Pharmacodynamics, Cardiovascular

Project 1	**MODELS FOR PARATHYROID DEVELOPMENT DISORDERS**	
Key Words (max. 5 words)	Parathyroid, parathyroid hormone, calcium, development	
Expected duration of the project (yrs)	Five Years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	During the early development of a human embryo, in approximately 1 in 4000 people, anomalies can occur in the structures that ultimately become the head and neck. This can lead to a condition in which patients have defects and therefore decreased function of their parathyroid glands. The parathyroid glands are located in the neck and produce a hormone responsible for regulating the levels of calcium in the blood. Therefore, the decreased function of the parathyroid glands leads to a decrease in the levels of calcium. This dysregulation of calcium can produce a number of symptoms including epilepsy, involuntary muscle contractions (tetany and cramps), cataracts and abnormal skeleton and tooth development. Despite this, the way the parathyroid glands develop, and the influence of environmental factors, such as diet, on these conditions are still poorly understood. Furthermore, better therapies are required to treat the symptoms of these patients with parathyroid	

	development disorders. We plan to address these issues by developing new mouse models, using information gained on the genetics of patients suffering with the abovementioned condition to gain further insight into parathyroid gland development. We also plan to utilise these, and current, mouse models to test the effect of different calcium levels and vitamin D in the diet, and also to develop and test the effect of new therapies.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary potential benefit of this work is increased knowledge of the mechanisms responsible for controlling calcium levels, parathyroid gland development, and diseases resulting from disruption to these. We believe this increased knowledge may yield new diagnostic tools (e.g. the identification of novel genes causing parathyroid development disorders) and identify new therapies (e.g. calcium regulators shown to decrease blood calcium levels in the mouse models). The secondary potential benefit relates to the value of the results to clinicians, as further understanding of these disorders will have clinical impacts in prevention and treatment of patients with these disorders. This may include screening of genes we have discovered to be involved in parathyroid development disorders, which will have an impact on clinical diagnosis.
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 enough 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 the 4,000 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 parathyroid development disorders in mice, are likely to be causative in humans. Over the five-year duration of this licence

	we expect to use no more than 16,000 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 adverse effects of mice with decreased parathyroid gland function are expected include symptoms similar to that of human patients, and in the worst case include cataracts, cramps, involuntary muscle contractions and tetany. Similar effects are likely to be seen by mice on a low calcium or vitamin D diet. There may also be side effects of any drug given including weight loss and dehydration. There may also be adverse effects occurring do to protocols we are performing, this includes singly housing mice in metabolic cages for up to 5 days, after which male mice may not be 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 culled immediately in a humane way. These steps include careful weekly monitoring of mice for visible signs of distress, housing mice individually for the shortest timeframe possible to get scientifically meaningful results, and exposing singly housed males to soiled bedding to maintain exposure to the scent of previous cagemates. 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 culled 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	The parathyroid glands produce a hormone called parathyroid hormone, which maintains the body's calcium levels by targeting other organs including bone and kidneys. As this represents a whole-body system, it is not possible to investigate disease development and assess potential treatments without the use of live animals. 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 parathyroid tissue 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. The use of imaging also allows us to monitor the growth of the parathyroid glands in one mouse over time, therefore reducing the total number 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 and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Only mice will be used in our studies and were chosen as they represent the lowest mammal, displaying a sufficiently similar parathyroid system, and genetic similarity to humans. We have previously established and characterised mouse models for use in this study and further models of mouse parathyroid development disorders will be established using techniques and facilities that we have readily available, and prior experience with. 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 drug delivery.

Project 2	**Ghrelin and the control of insulin secretion**	
Key Words (max. 5 words)	Ghrelin insulin diabetes	
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)	The characterisation of the role of ghrelin in glucose homeostasis in diabetic rodents	
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)?	Ghrelin is a hormone which decreases insulin secretion. It is possible that, by inhibiting the action of ghrelin, the insulin response to a meal may be enhanced in diabetic patients, helping them to avoid high blood sugar levels, and the diabetic symptoms associated with these.	
What species and approximate numbers of animals do you expect to use over what period of time?	This project will involve up to 80 rats and 360 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	It is possible that some of the novel potential medicines used in this project may cause adverse effects. This risk will be mitigated using pilot studies. The studies will use obese and diabetic animals, and this may cause some adverse effects for the animals,	

happen to the animals at the end?	in the form of increased thirst and urination, and potentially increased skin complaints. These effects will be mitigated by increasing the availability of water, and the amount of bedding, and by increasing the frequency of cage changes. There is also a slight risk of skin-complaints in obese rodents. Close observation will identify where this is an issue, and animals will be removed from studies and killed humanely to prevent further suffering. There is a low risk of adverse effects and injury during procedures carried out on animals (such as administration of substances by injection or oral gavage, oral gavage of glucose, and blood sampling). In these circumstances, the impact of the procedure on the animals, and on the ability to generate useful data, will be assessed. If either the animal has suffered undue harm, or the experiment is compromised, then the animal will be removed from the experiment either permanently (in which case the animal will be euthanised by a schedule 1 method) or temporarily, if the animal is expected to recover.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As much as possible in vitro cell based studies will be used instead of animals. However, glucose metabolism is controlled at a whole-animal level, and so animal studies will be required to test hypotheses raised in in vitro and ex vivo studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise numbers, studies will be carried out so that each animal acts as its own control. This means that individual animals will undergo more procedures. However, the procedures used are relatively un-invasive, and in addition the group-size is decreased (since between-animal variance is expected to be greater than within-animal variance) so fewer overall procedures are expected.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	The rodent models proposed are well validated as giving translationally-meaningful data, which predicts effects in man. Where possible, mice will be used rather than rats, but in some cases it may be necessary to use rat models of diabetes, since these are considered the gold-standard model for human

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	islet.

Project 3	**Transporters/receptors and tissue metabolism**	
Key Words (max. 5 words)	Insulin, muscle, metabolism, endocannabinoid, amino acid	
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
		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)	This project aims to understand the role of played by membrane transporters/receptors in the regulation of hormone and nutrient-induced signalling as it relates to control of key cell/tissue functions (e.g. fuel storage and metabolism). The work will provide insight and understanding of how defects in these signalling pathways contribute to pathogenesis of metabolic and nutritional-related disorders such as insulin resistance and muscle/protein wasting.	
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 work that will be done under this project aims to explore mechanisms that contribute to the pathogenesis of insulin resistance/Type II diabetes – conditions closely associated with obesity and aging. Over-nutrition is a significant player in the development of these conditions, which, in large part, progress as a consequence	

	of nutrient-induced metabolic dysfunction. The project aims to delineate the role of cell surface nutrient transporters/receptor proteins and the signalling networks that these proteins link to in an attempt to understand how nutrient-induced defects in metabolism develop. By doing so it is hoped that the project will identify novel molecular targets whose pharmacological manipulation may either help to ameliorate and/or better manage obesity and age-related metabolic disorders – especially those linked to energy metabolism and cell growth. It is important to stress that such disorders are not limited to humans and that obesity-induced insulin resistance and diabetes is increasingly becoming a major veterinary issue. Consequently, the outcomes of the research associated with this project should ultimately be of benefit to both humans and animals.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will utilise small rodents (i.e. mice and rats). We propose to use no more than 1750 rats and up to 5250 mice (these are the upper limit numbers) over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The overall severity limit of this project is considered “moderate”. However, in most cases it is anticipated that animals will not be unduly stressed or in discomfort. For the most part animals will be placed on select diets or hormone/drug treatments. These treatments may be accompanied by changes in weight. Those losing weight may display signs of lethargy. The hormones and drugs may cause some derangement of normal physiological function leading to loss or gain in body weight. Additional effects of drug administration will be drug dependent (e.g. steroids such as dexamethasone may cause lethargy or adrenaline may cause hyperactivity). The project may make use of genetically altered animals bought in from a commercial source or bred in house for continued used in this project. Whilst some of the procedures will involve drug

	administration to either acutely induce diabetes or sustained administration for specific periods to affect whole body energetics the animals will all be humanely killed at the end of the proposed studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our present understanding of the roles that membrane receptors/transporters play in the regulation of insulin action, growth pathways and fuel metabolism stem largely from cell-based work. This approach will continue to provide an important platform for future studies. However, there is an important need to not only test and validate the physiological relevance of our cell-based findings but, in some cases, to utilise more appropriate experimental (animal) models to test certain hypotheses.
2. Reduction Explain how you will assure the use of minimum numbers of animals	I will call upon 30 years of research experience and make use of the available literature in areas allied to my research interests to ensure best practice and make use of appropriate statistical power analysis software that will allow me to estimate the minimum number of animals that are needed in our research to give statistically meaningful results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	This research will specifically make use of mice and rats. 99 percent of genes in humans have counterparts in the mouse and consequently studying how expression of rodent genes or their encoded protein products impact on tissue metabolism/insulin sensitivity may potentially shed important insights into the pathogenesis of cardiovascular disease and diabetes in humans. Given their small size and short generation times using mice and rats offers significant advantages. Furthermore, since rodents have been used widely in research for decades, researchers have accrued a detailed understanding of their biology and genetics and

	developed numerous research tools/techniques to study them. We strive constantly to refine our protocols to ensure minimal suffering to the animals being used. For example, rather than employing high intensity exercise training protocols (e.g. treadmill running or swimming) to explore the benefits of exercise on whole body insulin sensitivity we propose to utilise a voluntary wheel running approach that rewards animals with food when they have run a certain time/distance. This approach makes use of the animal's natural instinct to wheel-run and avoids inducing stress that may otherwise be encountered by other protocols. Animals being used as part of this research programme will be regularly monitored by staff within our animal unit with particular attention to food, water intake, coat condition and eye colour and general demeanour. Where there is concern animals will be carefully assessed and, if need be, we will seek appropriate veterinary advice regarding treatment/maintenance of the animal or take steps to humanely terminate the procedure.

Project 4	**Proteolytic cleavage of the LDL receptor**	
Key Words (max. 5 words)	Cholesterol, Low density lipoprotein receptor (LDLR), protease, inhibitor	
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)	Cardiovascular disease kills one in three people in the UK. One of the most significant risk factors for cardiovascular disease is elevated levels of low-density lipoprotein (LDL) cholesterol – often referred to as 'bad cholesterol' – in the blood. The amount of LDL-cholesterol in the blood is controlled by its uptake into liver cells. On the surface of the liver cells is a specific protein, the LDL receptor, which binds the LDL-cholesterol. The number of active LDL receptors on the surface of liver cells is the single most important factor in regulating the amount of LDL-cholesterol in the blood. We have identified that the LDL receptor is cleaved into two smaller fragments by an enzyme. These smaller fragments of the LDL receptor are unable to take up the LDL-cholesterol into the cells. Decreased LDL uptake results in increased circulating LDL, which can lead to high cholesterol levels. The overall aim of this project is to test the hypothesis that cleavage of the human LDL receptor regulates LDL receptor function and	

	hence plasma LDL cholesterol *in vivo*. We will use mouse models to determine whether regulation of the activity of the enzyme may be a novel mechanism to lower the concentration of LDL-cholesterol in the blood.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our research will lead to improved knowledge and an advanced understanding of the fundamental science underpinning regulation of LDLR and cholesterol metabolism. This understanding will provide an advantage to academic researchers worldwide seeking to develop novel drugs to lower plasma cholesterol. This could have a significant global impact by potentially enhancing the quality of life, health and well-being of individuals affected by high levels of LDL cholesterol. Our research also has potential in the development and commercialisation of novel therapeutic treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 670 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?	*Severity category* Moderate: while the severity category of the surgical procedure is moderate, the severity category of the majority of the work contained within this application is mild. *Adverse effects* 1. Slight and transient pain during ear-notching required for genotyping of animals generated from conventional or cross-breeding of animals. *2. Weight gain and skin changes from feeding the animals a high-fat/high cholesterol diet (Western diet).* 3. Pain from the wound after the implantation of osmotic mini-pumps. Most animals undergoing any dietary or surgical interventions will be sacrificed under terminal anaesthesia. Remaining animals will either be kept

	alive for breeding and maintenance for continued use in this project or other relevant projects as authorised under this license or sacrificed by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have performed extensive alternative studies to confirm that cleavage of human LDLR reduces cellular LDL uptake. Having performed these extensive studies we believe that we have exhausted the current available systems and have significant data to warrant confirmation of our findings in an animal model. We now need to confirm the effects *in vivo* to provide proof of principle before embarking on exploring possible therapeutic targets of this pathway. We have been unable to identify an alternative to an animal model to allow us to verify our findings, however if any alternatives became available during the course of the project, we would implement these into our studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have ensured that we have the appropriate control and experimental groups to enable us to make appropriate conclusions from our experiments and have rationalised our experimental design to ensure that we have a specified programme of work with clearly defined objectives to limit the number of animals we require. In addition, we have also performed power calculations, using our cell-based work as an informative measure of effect size, to ensure that the study is appropriately powered to give meaningful, statistically relevant, data while ensuring that we use the minimum number of animals in our work.
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	*Species* As murine LDLR is not cleaved it is necessary for us to use mice expressing human LDLR. This model is the most relevant model that currently exists that will allow us to test our hypothesis *in vivo* a crucial step in determining whether this mechanism is physiologically relevant for the treatment of high cholesterol.

(harms) to the animals.	Any dietary modulations have been restricted to the shortest time period possible, while the surgical interventions have been designed carefully with the administration of sufficient and appropriate anaesthetics and analgesics so as to cause minimal suffering, distress or lasting harm to the animals. During surgery and recovery animals will be monitored closely to identify any signs of distress or harm.

Project 5	**BONE MARROW ADIPOSE TISSUE AS AN ENDOCRINE ORGAN**	
Key Words (max. 5 words)	Bone marrow, adipose tissue, calorie restriction, obesity, metabolic health	
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)	This project seeks to determine the impact of bone marrow adipose tissue (MAT) on human health. Scientists first noticed that our bone marrow contains fat-storing cells, called adipocytes, over a century ago. Having fat in our bones might strike you as unusual, but it is not: MAT makes up to 70% of bone marrow volume in healthy adults, suggesting a role for MAT in normal human physiology. MAT further increases in conditions of altered skeletal or metabolic health. For example, MAT increases during osteoporosis, suggesting that MAT might promote bone fragility. Perhaps most bizarrely, MAT formation increases during states of caloric restriction (CR). This is in stark contrast to adipose tissue elsewhere in the body, called white adipose tissue (WAT), which is broken down during CR to supply energy. CR has numerous health benefits, including decreased risk of	

	cancer and cardiovascular disease. MAT also increases following treatment with anti-diabetic drugs, which, like CR, enhance insulin sensitivity. In contrast, recent research finds that MAT can also increase during obesity, a disease associated with adverse metabolic health. These observations suggest that, like WAT, MAT might directly impact metabolic health. However, unlike WAT, little is known about the biological function of MAT. To address this major gap in knowledge we will pursue the following objectives: 1. Determine why MAT expands during CR and obesity. 2. Determine if MAT expansion contributes to metabolic effects of CR and obesity.
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 research will enhance our understanding of MAT, potentially revealing new insights relevant to human metabolic and skeletal health. This enhanced understanding could improve medical practices and allow development of new drugs. For example, MAT is an emerging clinical biomarker for increased fracture risk. Our research will reveal if MAT also affects metabolic health; if so, MAT might be associated with altered risk of metabolic diseases. Such knowledge would better inform MAT's use as a clinical biomarker. Moreover, if MAT directly impacts metabolism then it might be a viable therapeutic target for treating diseases such as diabetes.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use various strains of common laboratory mice, because these are well-characterised, reliable models of human health and disease. Over this 5-year project we expect to use up to 2800 mice; approximately half will be used in breeding programmes. We will use as few mice as possible to answer our questions, aiming to maximise the amount of information obtained per animal to help realise the potential benefits of our research.
In the context of what you propose to do to the animals, what are the expected adverse	Our proposed interventions include CR, obesity, adrenalectomy, and surgery to implant devices for substance delivery. CR causes decreased body

effects and the likely/expected level of severity? What will happen to the animals at the end?	weight and minor bone loss, but not to a pathological extent. Obesity (via high-fat diet feeding) is expected to cause metabolic disruption (e.g. insulin resistance, diabetes), but this isn't expected to markedly impair the health of the animal. Very little mortality is associated with device implantation, while mortality rates from adrenalectomy are low (<10%). In all studies, we carefully monitor mice to ensure that moderate severity levels are not exceeded. Mice are humanely euthanized at the end of each study, once we have gathered the necessary information.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	CR induces interconnected physiological effects on diverse tissues, including the brain (e.g. appetite), the skeleton (e.g. MAT expansion), WAT (e.g. decreased lipid storage), and muscle (e.g. increased fat oxidation). Obesity also causes numerous systemic metabolic effects. Studying such effects is not possible *in vitro;* hence, there is no way to replace the insights gleaned through animal studies. We extensively analyse animal tissues taken post-mortem and human tissues from clinical studies. These approaches support and complement our investigations in live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use statistical power calculations (based on published research from others and ourselves) to determine the minimum number of mice needed to robustly measure meaningful results of our experiments (e.g. dietary or genetic manipulations). To further reduce the overall number of animals required, we: • Use inbred mice to reduce inter-animal variability. • Where possible, use a multi-factorial design to increase statistical power. • Use imaging techniques (similar to those used in humans) in live animals, allowing repeated non-invasive measurements within a single animal, increasing statistical power. Effects of treatments are based on comparison with

	appropriate control groups. Study design is based on current best practice and, where necessary, following discussion with statisticians. Regular meetings within our research group ensure that maximal data is obtained from animal studies. We also allow other scientists to analyse some of our post-mortem animal tissues, thereby providing valuable insights into other fields (e.g. cancer, immunity) and reducing the need for additional animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mouse models best suited to address the experimental question, e.g. obesity-prone strains of mice are used for obesity studies. Mice with genetic/surgical/pharmacological modifications (e.g. to prevent MAT formation) are the most refined models possible for addressing MAT formation and function. Throughout our studies we strive to minimise suffering. For surgical procedures, appropriate anaesthesia, analgesia, postoperative care and aseptic techniques will be used. Drugs will be administered at non-toxic dosages. CR studies use a micronutrient-enriched diet to avoid malnutrition. For some metabolic analyses we use new low-stress procedures that minimise suffering while maximising the amount of information obtained per animal, e.g. non-invasive imaging for fat mass determination. We progressively develop and refine our methods. For example, our metabolic analyses begin with exploratory methods (e.g. insulin tolerance tests) to assess if experimental interventions have major effects on broad outcomes (e.g. “insulin sensitivity”). Only in the presence of a clear effect are more in-depth methods used, such as using infusions of labeled nutrients (e.g. glucose) and tracking their fate in a living animal. Such in-depth methods allow us to better determine the mechanisms underlying major effects. For such methods, the use of any invasive (e.g. surgical) techniques is discussed with colleagues performing similar work locally and across the country. We will tailor monitoring systems to each

	model and apply strict humane endpoints to minimise suffering. Finally, training and good practice is encouraged through group meetings and regular discussions with the NVS and key staff.

Project 6	**In vivo profiling of novel drugs**	
Key Words (max. 5 words)	Pharmacokinetics, Pharmacodynamics, Cardiovascular	
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)	The overall aim of the project is to give support to the discovery phase of research projects for external clients, ensuring that potential medicines with suitable pharmacokinetic — what the body does to the drug (PK) and pharmacodynamics — what the drug does to the body (PD) properties can be selected for further development to treat human disease effectively. PK is investigated by studying how potential medicines are absorbed and distributed in the body as well as how they are broken down (metabolised) and excreted. PD is investigated by the affect a compound has on the cardiovascular system. Typically a medicine that is given orall is dissolved in the gut, absorbed into the blood and then the circulated around the body. It may be metabolised, usually in the liver, and it, and/or its break down products (metabolites) excreted in urine and faeces. Some medicines cannot be given orally: for example, due to poor absorption from, or breakdown in, the gut	

	and another route must be used. Other medicines, such as those given by inhalation, do not need to go into the blood stream in order to elicit the desired pharmacological response and/or are more effective when directly delivered to diseased tissue. Confidence in predicting pharmacokinetic properties in man is gained by studying the action of the potential medicine in more than one animal species. The information gained from studies carried out under this licence will help to; • Understand and then improve the way the compound is given so that there is sufficient information available to treat the disease effectively. • Understand and then improve the length of action of the compound so that it is likely to stay in the body long enough when given in a dosing routine that is easy for patients to use. Potential clients are required to disclose any previous in vivo investigations done on their compounds, to avoid any unjustified duplication of procedures.
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)?	Work under this licence will assist our clients in selecting compounds with an expectation of suitable properties in man and thus reduce the risk of exposure of human volunteers and patients to compounds that would be unsuitable as therapies. The work will enable clear decisions by our clients to progress or halt compounds at key project milestones. Data generated will assist in designing appropriate regimens and limit unnecessary use of animals for pharmacological and toxicological studies.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats, mice and guniea-pigs will be used on this licence. It is expected that no more than 9300 rats, 12300 mice and 600 guinea-pigs will be used over the 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 severity? What will happen to the animals at the	Adverse effects (eg piloerection, mild sedation and salivation) are those associated with routine routes of administration, sampling and those associated with general anaesthetic. In these cases, the likelihood of occurrence is estimated at <1%. The administration of test compounds substances will have the potential to

end?	affect all animals. Close monitoring and use of pilot studies, will help to keep the incidence of adverse effects to a minimum. Animals will be humanely killed at the end of a protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no non animal tests (in vitro - in glass) that completely mimic and predict many key aspects of the work described in this licence eg: does the drug get to the target tissue or organ and does it affect blood vessels. These aspects are often the result of the interaction of many individual biological processes and these interactions cannot be reproduced by in vitro (non animal) alternatives. For most organs there are currently no in vitro models that predict organ clearance and only an intact animal can provide an adequate integrated system. Consequently, animal testing is essential to achieving the overall objective of this licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Certain properties of drugs can be studied using a range of in vitro (‘in glass’ or ‘test tube’.) and in vivo (‘in life’ or ‘in animals’) studies. It is standard practice to conduct the major proportion of testing in vitro using cells and tissues obtained from humans or animals. This strategy makes a vital contribution towards minimizing animal usage. In our experience, fewer than 10% of compounds tested in vitro are progressed to in vivo studies. In addition to in vitro testing, the properties of compounds are also predicted based on knowledge of their structure (‘in silico’). Whilst in silico (using computers) predictions or in vitro studies can provide a wealth of information on individual body systems, eventually studies in living animals are needed. Animals offer suitable models to replicate the interplay between different processes that can influence the disposition (what happens after it is given to the patient)of the potential medicine. Once in vivo work is deemed necessary, there are a number of approaches adopted in order to minimise the numbers of animals used;

	• Typically group size is 3 rats • Refinements in sampling in mice, reducing overall numbers required. • Consideration of statistical analysis, to use the smallest group sizes possible, yet maintain adequate precision. In pharmacodynamic studies the minimum number of animals required to detect effects of scientific interest will be determined using sample size calculations.
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 colonies. Most studies will be conducted in rats; this is supported by in vitro testing which shows the relevance of this species to man. It is necessary to use other rodent species, for the purpose of this project mouse, if these species are more relevant to man for a particular research project or if more than one species is needed to build further confidence in predicting to man. Animal suffering will be minimised by the following; • Competent personnel will perform all studies on this project licence and adverse effects resulting from regulated procedures 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. • A refinement in sample analysis has led to the reduction in total blood volumes required, now typically 2Opl sample size. Thus reducing the burden further.