Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 5

Projects with a primary purpose of: Basic Research – Gastrointestinal System including Liver

Project Titles and keywords

1. **Chromatin dynamics in intestinal epithelium health**
    - Intestine, nutrition, chromatin, gene regulation

2. **Characterization of novel diagnostic and therapeutic targets in metabolic pathologies**
    - Glucose, Hormone, obesity, Diabetes, fibrosis

3. **Control of Liver Glucose Metabolism**
    - Type 2 diabetes, Glucokinase, Anti-diabetic drugs

4. **Innate immune pathways in pathological and protective immunity in the gut**
    - Immunology, microbiota, gut, IBD, Crohn's Disease

Project 1	**Chromatin dynamics in intestinal epithelium health**	
Key Words (max. 5 words)	Intestine, nutrition, chromatin, gene regulation	
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
	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)	In the gut, a layer of cells (the intestinal epithelium) is essential for the uptake of nutrients and fluids. This layer of cells also protects the body from invasion by microbes, which are sometimes present in the food and which could cause disease (microbes that cause disease are called pathogens). The cells are assisted in these functions by an army of immune cells that are either resident within the tissue or access it when needed. These immune cells protect the body by inactivating and destroying pathogens. The intestinal epithelium layer of cells is constantly renewed and this renewal process is essential for its operation. Defects in gene expression (explained below) in gut cells can lead to aggravating diseases, such as inflammatory bowel diseases (IBDs) and colon cancer. These diseases are sometimes difficult to diagnose and treat. Furthermore, no cures have been developed for IBDs. IBDs are significantly on the rise, especially in developing countries. IBDs are currently estimated to affect more than 100 000 people in the	

	UK alone. This increased occurrence may be linked to changes in lifestyle such as diet. We hypothesise that IBDs are linked to defects in gene expression in the gut's intestinal epithelial cells. The function of our cells depends on the proteins that are active in them. Proteins can be thought of as molecular machines with a whole variety of roles in the cell. The instructions to make all the proteins needed by a cell are encoded in DNA; a gene contains the DNA sequence to make a protein. The DNA sequence (which can be seen as a long string of letters) is read and used as a template to make a specific protein. This process of reading the DNA to make a protein is called gene expression. Our research aims to illuminate how gene expression in intestinal cells is regulated by the packaging of DNA within cells and how the content of the gut (the food we eat) affects this packaging and, therefore, how genes are turned on and off. We will relate this to changes in gene expression that occur in IBDs.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our studies have the potential to reveal and illuminate causes of inflammatory bowel diseases and to identify new diagnostic markers and therapeutic interventions, possibly leading to the development of new treatments and dietary regimes.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 8650 animals/ 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The vast majority of animals we use will exhibit no or only little distress (>5000 animals). These animals are kept for breeding and maintenance of genetically modified mice (mice where the genome has been altered so they express genes differently compared to normal mice). Less than 10% of total usage of mice (800) is expected to be subject to experiments, which may lead to moderate effects, such as diarrhoea and weight loss. These mice will be closely monitored and will be humanely killed if symptoms worsen (e.g.,

	more than 20% weight loss). Animals subjected to experimental procedures such as these experiments leading to inflammation of the gut will be killed at the end of the studies in a humanely way and tissues harvested for analysis. Some mice will also be provided with different diets (for example a diet that contains a lot of fat) to see what sort of effect this has on gene expression in the gut, which will be examined after the mice have been humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The intestinal epithelium is a complex tissue where epithelial cells are in close contact with immune cells and bacteria that normally reside in the gut without causing any harm (called commensal bacteria). While aspects of this tissue can be mimicked to some extent in vitro (artificially in the lab, not in a living system) using cell culture methods, we need to study the tissue in the animal to fully understand how it functions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We employ mathematical (statistical) analysis, e.g. of small scale pilot experiments, to ensure we use the minimum number of animals in each experiment to allow us to still make solid conclusions but to minimise suffering and the number of animals used. For this, we are assisted by a dedicated biostatistician.
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 the mouse, which is a standard animal of choice for medical research, especially research exploring gene function in mammalian animals. We use mice because particular kinds of mice are available or can be generated where specific genes (e.g., genes linked to intestinal diseases) are altered, removed or mutated. The mouse genome (its DNA blueprint) is fully sequenced and we can build on a huge body of literature and knowledge. Genome sequencing has revealed that the majority of human genes have equivalents (homologues) in mouse with fewer than 1% of human genes lacking homologues in the mouse. Mice are relatively inexpensive to

	breed and to maintain. The proposed experiments have been extensively used and refined by us and others. Before starting any *in vivo* (in the animal) procedures, detailed *in vitro* (e.g. in cell culture) and *ex vivo* (using tissue) experiments will have given important insights into dose, timing and relevant parameters. This will allow us to refine and reduce subsequent work involving animals. Small pilot studies will allow us to optimise ways or working in genetically modified mice. This will avoid the use of inappropriately sized groups and unnecessary suffering due to any unexpected increase in susceptibility of the experimental group of animals compared with controls.

<table>
<tr><td>Project 2</td><td colspan="2">Characterization of novel diagnostic and therapeutic targets in metabolic pathologies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Glucose, Hormone, obesity, Diabetes, fibrosis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="9">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>X</td><td>Basic research</td></tr>
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<tr><td></td><td></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">The rising prevalence of type 2 diabetes (T2D) constitutes a major health problem that strongly correlates with an elevated incidence of obesity. It is estimated that almost 500 million people will suffer from this disease worldwide in 2030.

The communication mechanisms which regulate levels of certain substances in body tissues in response to environmental and nutritional demands is impaired in T2D patients.

The liver has a pivotal role in this commurication, controlling theassimilation of the products of digestion and the secretion of key molecules which regulate internal processes. Characterisation of these molecules is critical to understand the mechanisms leading to insulin resistance and the complications commonly associated with obesity and T2D, such as liver fibrosis, and to generate more selective and efficacious therapies.</td></tr>
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	We will use both computational and experimental approaches to identify the mechanisms and molecules involved in the communication between liver cells and other types of cell, in health and 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)?	The characterization of these molecules will provide valuable information about the hepatic and systemic metabolic homeostasis and may have potential diagnostic and therapeutic utility in metabolic diseases. In addition the information obtained in these studies will contribute to the advancement of knowledge in this area through the publication of findings in peer-reviewed journals and their dissemination between specialized and non-specialized audiences.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice: 2500/year
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Our protocols will be of a mild to moderate level of severity. In brief we will be inducing pathologies with administration of diets with high caloric value and/or chemical/genetic manipulation. We will then try to reverse disease using drug treatment, and/or alteration of diet regiment. Treatment and monitoring of pathologies will require the use of procedures similar to those used in human patients. Many of the experiments are ex vivo, in which case the animal is killed to obtain tissue. In the case of in vivo experiments, animals are killed at the end of the experiment, typically followed by further experimental analysis (e.i. anatomical or molecular). Animals will be killed by a humane method at the end of the project period. Animals exhibiting any unexpected harmful will be killed, or in the case of individual animals of particular scientific interest, advice will be sought from NACWO, NVS or the local Home Office Inspector. If the animal fails to respond to treatment or its condition deteriorates, it will be humanely killed.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The maintenance of normal blood sugar requires the coordinated interplay between tissues such as the liver, pancreas, the skeletal muscle, adipose tissues and immune system. Neuronal outputs from the brain in response to changes in hormonal signalling and nutrient availability also modify the net effect on blood sugar. Such complex interrelations cannot be reproduced in vitro and require a whole living organism. Although we do most of our work on cell lines and freshly isolated primary cells from animals humanely killed by Schedule I methods, we ultimately need to validate the effects on metabolic control in the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Several of the protocols that we use are designed in such a way as to obtain the maximum possible data from a single animal. For breeding genetically modified mice, where possible, we use strategies that maximise the use of offspring. In addition, when possible, our primary approach to express or inactivate genes in the liver will be the use viral or DNA vectors, avoiding the need of generating, breeding and using genetically modified mice. When appropriate, we will cryopreserve mouse lines that are not required for extended periods, rather than maintaining stocks. For most of the quantitative experiments, sample sizes will be set using careful statistical analysis. We will use the least number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from other published reports). Usually 6-10 animals per treatment group are sufficient to obtain the required 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	Mice are the lowest vertebrates in which genetic manipulation can be successfully achieved and where diabetes studies are well documented. In addition, the availability of transgenic mice provides powerful tools for examining these scientific questions. 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. Pain relief will be

costs (harms) to the animals.	provided as appropriate and as advised by the veterinary surgeon. Animals are regularly checked for unexpected adverse effects, more frequently following surgical procedures .

Project 3	Control of Liver Glucose Metabolism
Key Words	Type 2 diabetes, Glucokinase, Anti-diabetic drugs
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):

Type 2 diabetes (T2D) is characterized by a rise in blood glucose (sugar) which if not adequately treated causes damage to the eyes, kidneys, blood vessels and nerves. It is also associated with fat accumulation in the liver, a condition described as non-alcoholic fatty liver disease (NAFLD). Both dietary and genetic factors affect susceptibility to T2D and NAFLD.

There is currently a major need for better drugs for T2D. The commonest drug for T2D is metformin (Glucophage), which has been in use for over 60 years and has various limitations. Its mechanism of action remains incompletely understood. Recently there has been extensive effort at development of new drugs for T2D, such as the Glucokinase Activators (GKAs). The GKAs activate the first step in liver glucose metabolism (Glucokinase) and were expected to be a great improvement on metformin. Although GKAs were more effective than metformin during the first 4 weeks of therapy, they rapidly lost their effect after 4 weeks. The reason for this is unknown. The purpose of this project is better understanding why GKAs lose their efficacy on blood glucose control and also better understanding of how metformin exerts its effects on blood glucose through its action on the liver.

The objectives are: (1) Identify the changes in the liver that occur during loss of efficacy of the GKAs on blood glucose control in mice on a standard diet and on a “Western style diet” with high fat and sugar; (2) Identify the role of the glucokinase regulatory protein GKRP (generated by the GCKR gene) in the changes induced in the liver by the GKA drugs; (3) Establish the validity of the mouse as a model for

studying a common human risk variant in the GCKR gene for NAFLD; (4) Identify the effects of GKAs in this mouse model for the GCKR risk variant; (5) Advance understanding of the metformin mechanism in liver.

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

1. Understanding the changes that occur in the liver during GKA therapy will guide the development of better drugs with a longer-lasting effect. 2. If GKAs are found to have beneficial effects on the liver when they no longer lower blood glucose, this could enable their use for Non alcoholic fatty liver disease, a condition for which there currently are no drugs. 3. Understanding how the common GCKR gene variant interacts with diet in affecting liver fat will guide on potential benefits of GKA drugs dietary composition in patients with the risk variant. 4. Better understanding of the mechanisms by which metformin affects liver gene expression will help development of metformin analogues for T2D.

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

Mice up to 800 Rats up to 40 In 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?

The studies will involve feeding mice either the standard mouse diet or a “Western style” diet enriched in fat and sugar to resemble human diets and without or with the GKA drug incorporated into the diet for up to 20 weeks. The doses of the drug used are expected to cause moderate lowering of blood sugar in early treatment (during the first few weeks) but not in later treatment. We will monitor this by blood sampling for analysis of glucose, insulin and triglyceride (fat). If the blood glucose is low we will supplement the drinking water with glucose. The diet enriched in fat and sugar is expected to mimic the changes in blood and liver fat that occur in man in type 2 diabetes. At the end of the study we will collect the liver for histology, liver cell preparation, and detailed metabolic analysis. This will be done either under terminal anaesthesia (non recovery) or after euthanasia. We will also breed some mice with genetic alterations affecting their liver metabolism, but the effects of this will be mild. All the procedures are classified as mild, moderate or non-recovery.

Application of the 3Rs

Replacement

In our previous work that led to this project we used cellular models (rodent liver cells) to determine the effects of GKAs on liver gene expression. This work showed

that the GKA drug switched off the gene of glucokinase. It is now necessary to show whether or not the same mechanism occurs in an animal model and if so at what stage in the treatment it occurs, whether it is dependent on the diet and how it relates to glucose tolerance. An animal model is now essential to definitively show whether the changes that we have identified in the hepatocytes occur in normal physiology.

Reduction

The studies are carefully designed to ensure that the number of mice in each experimental group (without or with the drug treatment) is appropriate to allow us to detect significant changes of a size corresponding to a meaningful physiological response. Power calculations have been performed on data from previous similar studies to enable us to estimate the number of animals required for a particular measurement and change.

Refinement

The mouse is a well validated model for glucokinase function (because the protein is regulated by sugars in a similar manner as in man). This species also allows us to use genetically altered models such as GKRP deficiency and a GKRP variant that models the common human risk variant for NAFLD. The latter is a new model for a common genetic variation in the human population. The model of GKRP deficiency was developed previously. This model maintains normal blood sugar unless it is stressed by diet in which case it develops an increase in blood sugar. We will monitor blood glucose and liver markers as part of the study measurements. This will inform on any unexpected stress or altered liver function. We will monitor the mice carefully throughout all stages of the study.

Project 4	**Innate immune pathways in pathological and protective immunity in the gut**	
Key Words (max. 5 words)	Immunology, microbiota, gut, IBD, Crohn's Disease	
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 immune system at barrier sites must eliminate pathogens, whilst tolerating beneficial commensal bacteria. Alterations in the mechanisms that control immune and inflammatory responses at barrier surfaces can lead to infectious and inflammatory diseases and cancer. Inflammatory bowel diseases (IBD), encompassing Crohn's disease and ulcerative colitis, are chronic debilitating diseases of the intestine, with no known cure. Current treatments are mainly directed at suppressing the harmful immune response, but these do not cure the disease and are associated with serious side effects, including increased risk of infections. In this project we will use mice to study key pathways that the immune system uses to respond to infection and to the bacteria that inhabit the large intestine. We aim to better understand how these immune responses are controlled and define how these become deranged in infection, inflammation and cancer.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our work will lead to an improved knowledge of how immune responses are induced and controlled in the intestine. They will also increase understanding of how the immune system contributes to the function of a healthy gut and of how alterations in distinct immune pathways may predispose individuals to chronic intestinal diseases. In future, our studies may lead to new treatments designed to suppress pathological immune responses or to induce protective immunity against intestinal pathogens. These studies may aid the development of new antimicrobial approaches and vaccines for treatment of infectious disease as well as identification of new therapies for IBD. Disease models have been instrumental in identifying new pathways leading to the development of new drugs to treat IBD. In addition, because common pathways are activated in different types of inflammatory disease, our findings may help to discover new treatments for other inflammatory disease, such as type 1 diabetes, rheumatoid arthritis and multiple sclerosis. Our work is relevant not only to human health as chronic intestinal inflammation is also a problem in the livestock industry, and IBD affects domestic animals including cats and dogs.
What species and approximate numbers of animals do you expect to use over what period of time?	In this project we will use mice. We expect to use 60,000 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?	The majority of animals will be used for breeding and maintenance of genetically modified lines, and to act as donors for cells for use *in vitro* or *in vivo*. These animals are expected to undergo a sub-threshold or mild level of severity. A proportion of animals (~20-25%) will undergo procedures with a moderate severity during induction of inflammatory bowel disease, intestinal infection, or induction of colon cancer. There are several adverse effects that may be associated with these different procedures, such as signs of intestinal inflammation like diarrhoea and some weight loss. Mice that have

	undergone such procedures will be monitored on a daily basis for signs of disease, discomfort or abnormal sickness behaviours. We will keep suffering to a minimal level by employing very well defined thresholds as end points and mice will be immediately killed by a humane method if they reach these endpoints. In addition, a very small proportion of animals (<5%) will undergo surgical procedures, usually in order to remove specialized immune tissues and again these procedures are of moderate severity. There may additional adverse effects here, such as a risk of infection at surgical sites, as well as localised pain and swelling at incision sites. All surgical procedures will be performed under anaesthesia and analgesics will be applied to mitigate the effects of surgery. All mice will be monitored on a daily basis for signs of infection, discomfort or abnormal sickness behaviours and will be immediately killed by a humane method if they reach well defined endpoints. All animals will be killed at the end of the procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we will utilise *in vitro* systems and cell culture techniques as much as possible, it is not possible to replicate the complexity of the host-microbe interactions that occur in the intestine using *in vitro* approaches. The intestine represents a very complex interface where a variety of tissue cells and immune cells interact with the bacteria that inhabit this environment cannot be recreated *in vitro*. It is not possible to grow all of these cells or bacteria *in vitro*, or to re-create all aspects of the tissue architecture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every effort will be made to reduce the number of mice used. Where possible we will develop *in vitro* approaches such as 3D cell culture systems. We will continue to optimise extraction methods to provide sufficient numbers of particular cell types with minimum usage of mice. Maximal use will be made of harvested cells and tissues. For example, spleen cells may be used as a source of immune cells for *in*

	vivo transfer as well as for biochemical analysis *in vitro*. Mouse requirements are reviewed regularly to avoid unnecessary breeding, and cells and tissues will be used from shared control animals by multiple researchers. We archive frozen tissue samples to permit analyses of novel factors without additional *in vivo* experiments, and we embryo freeze strains not in current use to prevent over-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.	The mouse is the most appropriate species as it is the lowest vertebrate species likely to produce satisfactory results. The type of inflammation that develops in mouse models of IBD and colon cancer are similar to that seen in human patients. Also, the wide availability of reagents to study and modulate immune responses in mice, coupled with the existence of many defined genetic mutants, facilitates the types of experimental manipulation necessary to fulfil the objectives of this project. Most of the models that we will use have been developed over the last 20 years and have been optimised for reproducibility and disease kinetics. Clinical scoring for models have been adapted to allow maximum scientific output whilst minimising suffering, and scoring is tailored to individual models. For the majority of models experiments will be terminated using clinical criteria, although the end point will vary in different models. Scoring schemes have clearly defined action points and humane end points to minimise suffering.