Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 21

Projects with a primary purpose of: Translational and Applied Research – Human Gastrointestinal Disorders including Liver

Project Titles and keywords

1. **Liver Regeneration and repair**
    - Liver, Regeneration, Cirrhosis, Cancer

2. **The role of mitochondria in regulating gut mucosal homeostasis**
    - Inflammation, Gut mucosa, Epithelial biology, IBD

3. **Immune regulation at mucosal and barrier surfaces**
    - Immunology, microbiota, gut

4. **Obesity Induced Liver and Pancreatic Disease**
    - Obesity, Nervous System, Liver regeneration, Pancreas, Perinatal programming

5. **Mechanisms underlying chronic liver disease**
    - Obesity, Nervous System, Liver regeneration, Pancreas, Perinatal programming

6. **Towards New Anti-Obesity Drugs**
    - Obesity, Drugs

7. **Gut development, homeostasis and disease**
    - Peritoneal adhesions, Hirschsprung's disease, maintenance of the intestinal tissues, enteric nervous system, development of therapies

8. **Implantation of biologically derived tissue scaffolds**

Project 1	**Liver Regeneration and repair**	
Key Words (max. 5 words)	Liver, Regeneration, Cirrhosis, Cancer	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The liver has a remarkable ability to regenerate and adjust its size in response to injury. This is best highlighted by our ability to perform live-donor liver transplantation; where a living donor's liver is divided in two, each half capable of re-growing to normal size in both the donor and the recipient. None the less this regeneration often fails in patients with liver disease. If we understood regeneration of the liver in the same detail as we do in other organs then we may be able to develop treatments for patients with liver failure. By performing a detailed examination of the regenerative cells in the liver and where they reside, the aim of this work is to understand what these differences are. We could then use this knowledge to aid the development of much needed treatments. The specific objectives of this project are to: 1. Use new cell tracing models to characterise the anatomical sites and cellular identity of the cells which preferentially regenerate the liver in health and disease models 2. Examine the effects of global and zonal liver injury and inflammation upon the zonally	

	defined regenerative cells 3. Examine the role of specific pathways in promoting and inhibiting regeneration as potential therapeutic targets 4. Explore the potential side-effects of carcinogenesis and fibrosis when acute regeneration is manipulated To achieve these goals we will identify and characterise the dividing cells in the liver. It is important that this process is assessed in a living adult mammal to reproduce the complexity of the situation in humans; where many cell types and signals interplay to control regeneration. This work will follow dividing cells in both healthy mice and mice exposed to chemicals which stimulate liver regeneration and a surgical model (like the removal of a portion of the liver performed in living human donors in a liver transplant). This work will then examine and test how various signaling pathways control these regenerating cells.
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 benefits of this work understanding of the process by regenerates in mice and man. In the future we, and the wider scientific community, aim to use this knowledge to produce therapies for patients of all ages with liver disease. These therapies may be in the form of transplanting the regenerative cells into patient's livers, or the development of medicines or devices to enhance the regeneration and/or function of the liver in patients dying of liver disease. It is also expected that by understanding how the liver regenerates we may also gain insights into how other adult organs regenerate which may help in treatment of a wider ranges of diseases in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 14,000 mice over 5 years for this project. The majority will not undergo any scientific procedures, but will be used solely for breeding and maintenance of colonies. A large proportion of the experimental mice will be observed in health following transgene induction.
In the context of what you propose to do to the animals, what are the expected adverse effects and the	Animals will be bred which allow us to identify regenerative cells, track them and to specifically target the pathways which are promising preclinical targets for regenerative therapy. The majority of mice will not

likely/expected level of severity? What will happen to the animals at the end?	undergo any adverse effects (less than the equivalent of a single injection by a needle) aside from ear notching for identification and genetic testing. Mice not directly participating in experiments will be kept in normal housing and humanely killed when they are no longer needed for breeding. We will often be able to use tissue samples from these mice after they are killed as normal controls. A proportion of the animals (approximately 20%) will be given short periods of liver injury, induced either through liver surgery (like that used in patients) or exposure to chemicals (like paracetamol) which can cause temporary damage to the liver. A smaller proportion of mice (<15%) will have a milder but more prolonged liver injury induced by other chemicals which can induce scarring and, in some cases, cancers of the liver. Tumour cells can also be grown in the laboratory. In some cases, we will treat animals with experimental chemical compounds as trial therapies and measure there effects upon liver regeneration or the development of scarring or cancers. This may involve adding substances to the food or drink or injection of substances. All experimental animals or those receiving treatments will be monitored closely by carefully trained staff, and any animals that display signs of being unwell or of distress will have measures instituted to relieve any suffering (such as pain killers) and if this is not quickly effective then the mice will be humanely killed. At the end of the study, all animals will be euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To ensure that a minimal number of experimental animals are used experiments will be carefully designed by experienced researchers. Where possible, cell culture models will be used to allow us to study regeneration, rather than a living animal. However, due to complexities of cell and signal interplay it will also be necessary to study this process in living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animal used in experiments will be carefully predicted based upon data from previous published work by ourselves and other scientists. In all cases this will ensure that the minimum number of animals required for the experiment to give us useful

	data will be used but also reduces the likelihood that the animal experiment would have to be repeated in the future.
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 continually refine the disease models we use to reduce animal symptoms and to improve the effectiveness of our models. This, in combination with the use of state of the art technology, allows this work to ultimately use fewer animals to generate meaningful and clinically relevant data, and reduce any ill effects for the animals themselves. This work will be performed by experienced doctors, vets and scientists and will closely monitor the animal's condition. We set strict limits to ensure that there is limited harm to the animals used.

Project 2	**The role of mitochondria in regulating gut mucosal homeostasis**	
Key Words (max. 5 words)	Inflammation, Gut mucosa, Epithelial biology, IBD	
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)	To investigate a new mechanism relevant to the human inflammatory bowel diseases (IBD, ulcerative colitis and Crohn's disease), focusing on the pro-inflammatory role of the gut mitochondria. The mitochondria **('batteries')** provide energy and play many key roles to maintain the health and function of living cells. The mitochondria within the lining of the large bowel however, are particularly exposed to many damaging factors in the gut. In health, these damaged mitochondria **('faulty batteries')** are effectively re-cycled or packaged away for safe disposal. In inflammatory bowel disease (IBD), we have shown that these protective processes do not work properly or are overwhelmed. The unhealthy colon therefore, leaks damaged mitochondria and their products into the internal environment of the cells and importantly into the blood circulation. Given that the mitochondria are evolutionarily derived from bacteria some 2 billion years ago, the mitochondria share and retain many similar properties with bacteria – including their ability to activate the inflammatory and immune system. Data in mice and humans have demonstrated that damaged mitochondria, which have escaped from the unhealthy colonic lining, become a **'danger signal'**: a	

	source of inflammation and may attract inflammatory cells to the site of leakage. Recent studies also show that blood level measurements of mitochondria DNA (mtDNA) are increased in unwell patients in intensive care units and that the leaked mtDNA into the blood circulation may actually directly contribute to the inflammatory process.
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 current study seeks to investigate the importance of mitochondria **'danger signal'** in driving the inflammatory response in IBD. Since there are a few approaches where we can block mtDNA inflammatory signalling, our data will be useful in finding individuals where this novel disease mechanism will be most relevant (**a stratified medicine approach**). If successful, this will form a part of a larger study to develop a selective, targeted clinical approach where the gut mitochondria inflammatory signalling blockade will be used as therapy in IBD.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. 6000 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We aim to study 3 key areas where the mitochondria are involved: the gut bacteria, the biological pathways at the gut lining (epithelium) and the gut immune/inflammatory response. We will use mouse as the biological model where we can manipulate (or change) these 3 areas and test their respective relevance using our colitis models. The maximum level of severity is set at moderate and our mice will be humanely killed at the end of our experiments. In our gut inflammation mouse models, it will be typical to expect diarrhoea, weight loss and sometimes, bleeding from the gut. Some models result in more rapid development of these signs and symptoms whereas others are more gradual in onset. We use a robust method to measure how unwell the mice will become. This involves daily weight measurements and assessment of overall mouse well-being, diarrhoea and the presence of blood in stools. We do not allow our mice to become significantly unwell (e.g. if they lose more than 20% of their weight or look visibly distressed). In these circumstances, these mice will be humanely killed. We have now more than 5 years' of direct experience in these experiments and the current technical staff in

	our facility are now well trained in these models.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Live animals are necessary because of the highly complex nature in the interaction between gut bacteria, the bowel lining and immune cells. There is not an alternative scientific model that can closely mirror this complex make-up. Furthermore, almost all gut cells that can be grown on a dish are derived from colorectal cancer cells and are grown in a highly artificial environment. They have their uses but do not accurately reflect the natural biology of the gut lining. Whenever possible, we will perform our experiments without using live animals, but final proof of our hypothesis needs to be tested in living systems in this setting.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use groups of 8-10 mice per experimental group. This is the minimum number calculated to provide the necessary answer to our experiments. We will have a clearly written plan that includes the necessary mice numbers before we actually conduct the experiments and aim to use as few animals as possible to achieve an answer.
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.	All our models are well described and have been used in high impact published studies. There is not one single model that will explain the diverse human IBD clinical presentation. Hence, in a wide ranging scientific approach to obtain the best data, more than one model is often needed. In order to minimise suffering however, we adopt these approaches below: • We aim to use the lowest possible concentrations of agents require to cause colitis. In new mouse lines where we have little prior knowledge of their potential sensitivity to these agents, we will titrate the level inducers starting from the lowest possible level. • We have a colitis risk score, with clear thresholds for our severity limits. This score is established within our animal unit and our technicians are very experienced with this. No mice will be allowed to breach the severity level set. • Some techniques involve direct administration of substances into the colon (large bowel) where we can limit the duration and exposure of potentially toxic substances. • In genetically modified mice that are likely to get

	colitis naturally (e.g. spontaneous rather than caused by a scientific trigger), they will be bred in a heterozygote state (e.g. possessing only one mutated gene instead of two) and kept in super clean environment to reduce this likelihood of the breeding mice to become unwell. For example, some of our mice can develop chronic colitis which are caused by normal gut bacteria. The onset of this is very much reduced in a clean environment and nullified when the mouse breeders are in a heterozygote state.

Project 3	**Immune regulation at barrier surfaces**	
Key Words (max. 5 words)	Immunology, microbiota, gut	
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. Similarly, psoriasis is a chronic inflammatory skin condition. Current treatments are mainly directed at suppressing the harmful immune response, which does not cure the disease. In this project we will use mice to identify host and microbial pathways that promote physiological immune responses in the intestine and skin and how these become altered in infection, inflammation and cancer.	
What are the potential benefits likely to derive from this project (how science could be	In the short term this project will improve our understanding of fundamental cellular and molecular pathways underpinning inflammatory diseases at	

advanced or humans or animals could benefit from the project)?	barrier surfaces. Such studies have already led to new treatments effective in the clinic. The new license is likely to lead to additional new treatments designed to suppress pathological immune responses or to induce protective immunity against intestinal pathogens. In addition, 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 a number of chronic inflammatory disorders in addition to IBD and psoriasis, including 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 82,000 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 experience no adverse effects and at most a mild level of severity. Some mice will be bred in germ free conditions (the absence of all bacteria), and some of these may experience some diarrhoea and enlargement of the abdomen due to an enlarged caecum. The majority of animals (~70-80%) will experience procedures with a mild severity. During induction of inflammatory bowel disease, cancer or psoriasis, some animals may experience a moderate severity (~20-30% of the total licence). These are expected to induce gut inflammation leading to some diarrhoea and some weight loss. Mice with psoriasis will have scaly skin on the areas affected. To replicate the effect of human metastatic cancer some mice given cancer cells may develop tumours in other parts of the body such as the lungs. In most cases however the tumour itself will not be metastatic. To assess the role of the

	particular immune system genetic changes in disease, a small proportion of mice (<5%) will receive irradiation treatment to remove their immune system and replace it with a new one, a procedure of moderate severity. 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 such as 3D cell culture systems as much as possible, it is not possible to replicate the complexity of the host-microbe interactions *in vitro*. This is particularly important for the intestine and skin as these complex interfaces with the environment and commensal and pathogenic bacteria cannot be recreated *in vitro*. There are no *in vitro* substitutes for assessing immune pathology and immune function at barrier surfaces.
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	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 psoriasis are quite similar to that seen in human patients. Also, the wide availability of reagents to study and modulate

measures you will take to minimise welfare costs (harms) to the animals.	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 monitoring for models has been adapted to allow maximum scientific output whilst minimising suffering, and monitoring 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. Protocols have clear humane end points and interventions to minimise suffering. To further minimise suffering we use aseptic technique to minimise the risk of infection, and provide analgesia to reduce pain. Our use of germ free animals, and germ free animals with specific bacterial species put back will inform on disease in the context of a very defined microbiome. This gives us a much better understanding of the effect of the microbiome on pathology, which is of particular importance in the diseases we are studying.

Project 4	**Obesity Induced Liver and Pancreatic Disease**	
Key Words (max. 5 words)	Obesity, Nervous System, Liver regeneration, Pancreas, Perinatal programming	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Obesity is the commonest cause of chronic liver disease and pancreatic dysfunction in developed countries. A proportion of affected patients will develop end-stage disease, with severe scarring and organ failure. These injurious processes may also lead to liver and pancreatic cancer, for which there are no current curative therapies. Fat accumulation (steatosis) in these organs can initiate a cascade of events leading to inflammation and scarring as a result of collagen deposition (fibrosis). Our main objective is to understand the role of the nervous system in obesity induced liver and pancreatic disease and identify manipulations of the nervous system that can help liver and pancreatic repair and regeneration. Our previous work supports that the nervous system can regulate liver repair. Normally, the repair/scarring process is well regulated which usually ends when the injurious agent is removed or	

	contained. The role of the nervous system in these complex processes of repair and scarring are uncertain. Moreover, given the similar embryological origins of the liver and pancreas, pathways that regulate liver repair may also be applicable to pancreas repair. We have shown experimentally that inhibiting the autonomic nervous system (ANS) causes release of liver resident stem cells to reduce liver injury and enhance repair. The elevated stem cell release is thought to protect from liver failure by replacing damaged liver cells (hepatocytes). As an objective, we will also study how maternal obesity and the perinatal environment affects offspring metabolism, as well as specific liver and pancreas disease outcomes. During the period of organ development and early life, cells and tissues are rapidly growing and they are exposed to external stimuli that can affect their normal development and future functionality. We have previously shown that maternal obesity increases offspring risk of developing obesity and consequently fatty liver and fatty pancreas diseases, which are the first steps toward liver cirrhosis and pancreatic failure. Furthermore, the maternal environment affects offspring response to stimuli during their life course. Maternal programming of offspring phenotype can also be affected by changes in the immune system, including activation and proliferation of cells implicated in liver protection.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The main benefits of results from our studies will be the translation of laboratory findings to a clinical setting through the identification of new molecular targets in liver and pancreatic disease, allowing for the development of new medicines to treat patients with these diseases. Furthermore, improved understanding of liver and pancreas perinatal programming will help promote the importance of health among fertile, pregnant and lactating women in order to prevent metabolic diseases and associated complications in subsequent generations. We will promote these findings through our patient focused campaigning

	charity and through government advice and the media.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 9000 mice and 2800 rats, to include genetically altered strains 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?	Most of the mice will be used for breeding purposes. The primary intervention that will be carried out will be based on diets that promotes obesity or highly palatable diet. Although the animals become obese, there is usually no associated animal distress and minor affections (e.g., difficulty moving, greasy skin) and obesity comorbidities (e.g., hypertension) could appear given the expected level of severity mild. Some animals (mainly those with surgery and organ damage) may experience discomfort (abdominal pain) or complications (such as infections, dehydration, hyperventilation or changes in natural behaviour) as a result of surgical procedures. All the animal exhibiting unexpected signs of distress will be humanely killed All animals will be provided appropriate pain relief and anaesthesia. With the perinatal programming model, the whole point is to induce obesity and study the impact of maternal or paternal obesity on offspring obesity. This development of obesity will not cause undue distress to the animals. These if present will be minor, such as decreased agility, greasy hair. Those animals in these protocols with changes in intestinal bacteria or supplemented with alcohol could suffer more distress. Therefore, we will score them for signs of distress as per established and enumerated scoring systems. Animals scoring highly on these scoring systems (equatinq with overt distress) 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	There are no existing experimental models using only cultured cells which can mimic the function of the whole liver, as this involves a complex interplay between many different cell types. The growth response of one type of liver cell is profoundly affected by close proximity to other types of cells, and so the

	use of primary cell cultures will not replicate the growth processes that occur in the living organism. Similarly, in those experiments aimed at treating diseased liver, the model of disease (e.g. cirrhosis or fatty liver) can only be developed in living organisms. This argument is similar for pancreatic function, and the necessity for whole animal modelling of pancreas disease. Thus, experiments will require whole animals. We shall however, always use in vitro or ex vivo culture approaches, to minimise or avoid the use of animals. Furthermore, the issue of life time, and ethical dilemmas of interveritional studies in pregnant women, makes rodents a more appropriate subject for the study of developmental programming influences.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our previous investigations using cell culture techniques allow us to minimize the number of animals required for experimentation in living organisms. Furthermore, our experiments incorporate factorial designs such that the information generated will be maximized from the minimum number of animals. We are also committed to maximising the amount of experimental information we obtain from each animal in order to minimize the final numbers that will be used as per the ARRIVE guidelines. According to the number of animals required for each experimental group, we will set the minimum number to achieve the best quality results for our scientific aims. We will determine the number of animals from each experimental group mainly based in our previous background, collaborators' experience with similar experiments and current literature on the field. Furthermore, we will also use power calculations to better determine the group sizes. Finally, we are also committed to maximizing the amount of experimental information obtained from each animal in order to reduce the final numbers that will be used. This compromise is in line with ARRIVE guidelines for maximizing all the information obtained from animal experiments and spreading it.

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.	Rodents show close homology with human physiology and their relatively short life cycle allows investigation focused on maternal nutritional and perinatal influences on the subsequent adult offspring. Furthermore, similarities between human and rodents in liver physiology and metabolism make them the most appropriate and refined model species for exploratory pre-clinical investigation. There is no reason that animals should experience undue pain and discomfort as a result of the procedures. Pain and discomfort will kept to an absolute minimum through good laboratory practice and there will be appropriate use of analgesic drug regimen. During surgical procedures careful monitoring as well as the use of aseptic techniques and perioperative and post-operative analgesia. Any animal exhibiting undue distress will be humanely killed.

Project 5	Mechanisms underlying chronic liver disease	
Key Words (max. 5 words)	Chronic liver disease, liver-gut axis, inflammation, metabolism, resilience.	
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)	Chronic liver disease is characterised by liver injury, inflammation, fibrosis, cirrhosis and tumor development regardless of the disease aetiology. The molecular mechanisms underlying the progression of chronic liver disease remain poorly understood. For this reason the therapeutic approaches for most chronic liver diseases, including non-alcoholic fatty liver disease and cholestasis, are scarce and in most cases ineffective. One of the main challenges of understanding the mechanisms underpinning chronic liver disease is that these pathologies are multifactorial and usually involve also disturbances in gut physiology and in the immune system. The main objectives of our work are: 1) Understand how the liver responds to injury and what are the molecular mechanisms mediating the progression of chronic liver disease. 2) Identify the key regulators of the inflammatory	

	response during the liver response to injury and the progression of chronic liver disease. 3) Define the role of the gut-liver axis communication in the pathogenesis and progression of chronic liver disease and how this impacts on the liver capacity to respond to injury. 4) Propose therapeutic strategies to improve the liver capacity to respond to injury and to counteract chronic liver 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)?	Liver disease is the fifth biggest killer in the UK and the number of cases/deaths increases yearly. To accomplish the aims proposed in this project will have a beneficial impact both on basic science and on translational levels. Our results will enable us to understand the mechanisms underlying chronic liver disease (essentially of non-alcoholic and cholestatic aetiology), which will lay the foundation to investigate potential therapeutic strategies to counteract the progression of liver disease. To translate our knowledge from experimental work to clinical practice will provide the background for future improved stratifying strategies to appropriately place patients into personalised drug- treatment programmes.
What species and approximate numbers of animals do you expect to use over what period of time?	Wild type and genetically altered (GA) mice will be used in this project during the next 5 years. We estimate that the total number of mice to be used in this project will be of 5.900.
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?	Most of experiments in the proposed project are expected to result in no more than transient, moderate pain and no lasting harm. The Surgical procedures proposed will be carried according to the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010) to minimise the suffering of mice. When appropriate, severity limits greater than mild will be controlled by use of general anaesthesia and analgesic treatments. We propose one severe procedure which is the bile duct ligation, after which a number of mice will be expected to show a sign of illness in response to this

	procedure. In this experiment, we will put in place a number of measurements to minimise the number of spontaneous deaths and to minimise the suffering and discomfort of the animals. To ensure this, mice will be closely monitored following a detailed behaviour/physical signs scoring sheet. Intermediate measurements include increased monitoring frequency and additional care measurements. When animals reach a specified clinical condition, they will be immediately humanely killed. This experiment will be performed only when it is essential to answer a specific research question. The length of the experiments will also be tightly adjusted to obtain significant and relevant scientific information and will vary depending on the research question to be answered.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The liver response to injury and the progression of chronic liver disease are multi-systemic processes that involve the communication between the liver and the gut axis. Moreover, inflammation is the hallmark of the pathogenesis of the disease. The complexity of these interactions cannot be reproduced in a laboratory cell culture setting. Moreover, nowadays it is not possible to monitor and regulate *in vitro* the cellular energy and metabolic responses undergoing during the liver response to injury. The immortalised cell lines available for *in vitro* studies show significant alterations in metabolism, accordingly with their immortalised phenotype, and are therefore not optimal to use when investigating cell metabolic pathways/responses. For these reasons, the relevance and applicability of the results obtain in isolated *in vitro* models may be limited due to the complexity of the *in vivo* interactions between different cell types and organs during the progression of liver disease. Such information will be only achieved by experiment using animals. As alternatives, experiments with human biopsy and primary cells isolated from mice will be

	employed wherever applicable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before performing the procedures detailed in this project we will ensure that the number of mice used will be the minimum to achieve our research objectives. Thus, previous statistical analyses will be performed in collaboration with our statistics department. Breeding colonies will be monitored carefully to avoid over-production of animals. We rely on our wide experience (more than 15 years) in performing animal work and especially in carrying out the experimental models proposed in this project such as partial hepatectomy, dietary interventions, and inflammation and cholestasis models. Our solid experience and our preliminary data will allow us to precisely design the experiments proposed in a way that we will obtain the maximum information with the lowest number of mice possible. Also, discussion with other researchers and extensive literature search will contribute to reduce the number of mice used. Finally, we will collect several samples; cells, blood and/or tissues from each single mouse aiming to maximize the use of each animal and minimize the suffering.
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 use of mice in this project is based on the fact that mice are the closest rodent to human in terms of genome, transcriptome and metabolism. Moreover, mice are the most extensively studied species for immunology research and, as opposite to rats, many genetically modified strains are already available. The relevance of mouse-based research to the human system is well established and therefore we anticipate that some of our results may be translated to the clinical setting. Unfortunately, most of the in vitro models cannot replace the multiorgan (gut-liver) interactions that we aim to study. Animals will be kept in social groups, and in enriched environments that allow them to behave in a normal

	manner. We don't anticipate deleterious phenotypes for the majority of the genetically altered mouse strains that we will breed, nevertheless, we will closely monitor these breedings, specially when new strains will be generated and animals will be humanely killed as soon as possible after a welfare problem is identified. We will always use the lowest severity procedure and model to achieve our scientific aims. Most of the treatments/procedures proposed may only cause a transient distress and no lasting pain. Diet modifications are not expected to cause any pain or distress is expected from the diet treatment. In the case of the procedures involving surgeries, we will follow the LASA guiding principles for preparing for and undertaking aseptic surgery (2010). Thus, we will proceed using the highest standards of aseptic techniques and high quality pre and post-operative care, including analgesia administration, measurements to avoid dehydration, maintenance of body temperature, wound treatment and ensuring appropriate food intake. Especially the surgical cholestatic model, a moderate model that can have adverse effects such as weight loss or ascites, will only be used to answer specific research questions and to obtain the maximum biologically relevant information. At all stages of our research, any animal showing pain or distress will be humanely killed. Each of the procedures proposed aims to answer a research question. After completion of each procedure, we will perform in depth analysis of the samples obtained and the results obtained will inform on the next experiments to be done. This will ensure the use of the minimum number of mice.

Project 6	Towards New Anti-Obesity Drugs
Key Words	Obesity, 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;
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 objective of the project is to understand how peripheral hormones (such as gut and liver hormones) affect the brain in a way that regulates food intake. This work could lead to new anti-obesity drugs. There is a huge clinical need for this research because of the global epidemic of obesity and diabetes. One key question that the research aims to answer is: ‘How does food activate reward-pathways in the brain and lead to so called ‘hedonic-feeding’?

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 work will help other scientists who are trying to understand how food intake and fertility are controlled by the brain. Ultimately, he work could lead to new strategies to treat obesity through dampening the desire for hedonic foods. It could also influence novel assisted reproductive therapies such as IVF.

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

We will use mice and rats. Over 5 years, we expect to use approximately 10,000 animals.

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 of the animals will have a genetic modification. Because we do not expect any adverse effects of these genetic alterations, the breeding and maintenance of these animals is considered a mild-severity procedure. Typically, these animals will be fed a pleasurable diet (high-fat/high-sugar) until they become fat. Inducing obesity in this way is considered a moderate-severity procedure. The animals will typically then undergo a procedure to investigate the effects of an anti-obesity drug-candidate on food intake. These drug treatments, and the important associated experiments to determine how the drug works, and identify potential side-effects, are considered moderate-severity procedure. Some animals will undergo routine surgery under general anaesthesia. This is required to; for example, implant a capsule under the skin to deliver the drug-candidate in a chronic way, or to remove the ovaries so that oestrogen does not interfere with the action of the drug-candidate. We will also perform brain surgery so that we can deliver drugs directly in to the brain, and even stimulate/image specific neurons using light-pulses (this is painless, the brain does not have pain-sensors). Because they involve general anaesthesia, these surgical procedures are considered to be of moderate-severity. The most important adverse effects will be the rare loss of an animal during surgery, or as a post-surgical complication. We have planned to minimise this outcome by ensuring excellent surgical technique and post-operative care. There are also obvious adverse effects of becoming obese, such as lethargy, and some loss of blood sugar control. There may also be unknown side-effects of our drug-candidates. However, these are naturally occurring hormones and our experience suggests that unexpected adverse side-effects are highly unlikely. All the animals will be humanely killed at the end of the experiment.

Application of the 3Rs

Replacement

The main reason that we need to use animals is that obesity is a system-wide disease. We know that hormones from the gut and liver affect the brain, and that the brain then influences eating behaviour. This multi-organ process cannot be replicated in a non-animal system.

Reduction

We will perform power calculations to ensure that the minimum number of animals are used, while still maintaining scientifically meaningful results.

We will perform non-animal experiments on our drug-candidates wherever possible. We will never perform a procedure on an animal unless there is good non-animal data to suggest that the drug-candidate might be effective.

Refinement

We will use mice for most experiments because they are the most refined species of animal that maintains hormonal axes that are similar to that of humans.

We will occasionally use rats because they respond to some reward-behaviours in a more human-like fashion than mice. For example, rats will chose to share a food-reward with its cage-mates whereas mice will not.

We will take a number of steps to minimize harm to the animals. These include using excellent and aseptic surgical technique. We also have a number of strategies to minimize pain, and these will be constantly updated on the advice of the named veterinary surgeon. For example, wherever possible, we will conduct experiments on terminally anaesthetized animals. We will also ensure the general welfare of the animals by regular monitoring.

Project 7	Gut development, homeostasis and disease.
Key Words	peritoneal adhesions, Hirschsprung's disease, maintenance of the intestinal tissues, enteric nervous system, development of therapies
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):

We aim to understand more about how the gut tube forms, and how it is maintained to function in the adult. We also aim to develop therapies that could treat patients with injuries or abnormalities in the gut. The therapies could be either substances or cells. We will use mice in which we induce the injuries or abnormalities to the gut, in order to test the 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)?

Our project will provide new information about how tissues of the gut tube develop and renew. Our project may also help develop to therapies for the treatment of injuries or abnormalities to the gut tube. Therefore, there are two potential benefits: an increase in scientific understanding, and the development of therapies for patients who suffer from gut tube abnormalities or injuries.

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

We will use mice. We may use up to 1500 animals during 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?

Some animals will have injuries caused by surgeries, and some animals will have abnormalities from birth. About 10-20% of the animals to transiently have pain, and transiently lose weight. A very small group of animals, i.e. 10 or less, will undergo more severe adverse effects which can include gastrointestinal syndrome, including weight loss and diarrhoea. At the end of each experiment, the animals will be killed humanely without pain.

Application of the 3Rs

Replacement

We cannot study the development and maintenance of the complex gut tube in a non-animal system. We cannot develop the therapies in humans as they may carry risks for the health of the human patients. We need to understand how the processes of development, maintenance and therapies work in the whole animals.

Reduction

Before we perform each study, we will consult with biostatisticians about the minimum number of animals to achieve our goals.

Refinement

We will perform some of our experiments over periods of up to 6 months where we will follow cells inside the animals using imaging techniques. This means that less animals will need to be used.

We will find out the optimal conditions for the treatments using small groups of animals before starting a large experiment.

The methods we will use have all be well described previously by other scientists and involve accepted models for the diseases.

Project 8	Implantation of biologically derived tissue scaffolds
Key Words	
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):

Recently, scientists and clinicians have replaced a patient's diseased windpipe (trachea) with one which had been made in the laboratory. This tissue engineered trachea restored normal function to the patient's lung thereby improving her quality of life. This impressive bio-engineered organ was achieved by taking a trachea from a patient who had recently died and removing all the cells. The remaining tissue was repopulated with stem cells taken from the recipient patient's bone marrow. The re-populated trachea, when transplanted, convinced the patient's body that it was a part of it thereby avoiding the danger of rejection which is a serious problem when undergoing normal organ transplantation.

As the demand to replace or renew old, injured or diseased tissue continues to increase, it is obvious that this demand cannot be met through organ donation alone . A tissue engineering solution may provide the necessary tissues or organs to meet this demand. The focus of research is geared towards using animal tissue or organs to create scaffolds that are identical to the tissue/organ that needs to be replaced in human patients. By seeding these scaffolds with cells (eventually from the patient) we hope to create functional tissue. Since we will use animal tissue/organs to create the scaffolds we will not compete with human tissue destined for transplantation. The animal derived scaffolds are made of collagen, similar to human collagen and its molecular makeup is preserved between species (e g between humans and pig/ sheep). Combining these scaffolds with the patient's own cells would result in avoidance of lifelong immune-suppressive drugs.

Our initial studies are focused on creating scaffolds from following systems: the respiratory system, the digestive system, the gut, the liver and skin and then seeding them with the most appropriate cells type, this might be a stem cells or any other type of cell.

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 ability to supply organs or tissues made in part from animal tissue for transplantation, which will not be rejected by the host, will transform the prospects of patients who currently suffer considerable poor quality of life due to failure of tissue function. The radical approach of decellularisation of donor tissues and repopulation of those tissues with host cells makes this type of transplantation or implantation possible. This will not only transform the lives and aspirations of the recipients but will also considerably reduce the financial burden to the NHS as these patients will no longer require as intensive or costly medical attention.

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

Where ever possible tissues/organs from donor animals (obtained from separate ongoing studies and where not possible, donor animals will be sourced) will initially be implanted into animals pigs (max 150), sheep/goats (max 150) or rabbits (max 150) (TOTAL no =450) as additional to or part of their normal tissue systems. This will allow us to analyse the function of the implanted tissues/organs without compromising their existing systems. Only tissues/organs showing appropriate function in these studies will go on to replace tissue systems. The number of animals required will vary depending upon the complexity of the organ or tissue being regenerated e.g few animals will be needed for the skin project but more will required for the liver and bowel.

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?

Possible adverse effects include infection, tissue rejection, pain (moderate), abdominal herniation and non function of implanted tissue. All these will be monitored and kept to a minimum, the overall expected severity level will be moderate. Since a full depth analysis of the transplanted tissue will be essential at the end of the experimental period all animals will be killed at this point

Application of the 3Rs

Replacement

If we can show that the tissues/organs that we have prepared are appropriately functional in a complete physiological system (the whole animal) their use can be progressed very quickly into human clinical cases.

Over the last 8 years we have been able to refine many of our experimental designs so that more work is done in the lab before moving to the animals. By developing these 3D cell models we can take very tiny amounts of cells from any animal and grow the cells over longer periods and look to see how they react to different stimuli e.g. growth factors. We also hope to be able to offer these models to other scientist as well.

Reduction

Proof of function can be established using only a few animals (typically 3-6) for each of the tissues/organs in the chosen species. We can also where possible, obtain tissue from unrelated studies at our research establishment to initially develop the scaffolds therefore avoiding killing additional donors.

Additionally we have been able to develop 3D models of cells which mimic what the tissue would look like in the animal, allowing us to obtain more information on how the cells and scaffold will behave to together in the lab rather than constantly relying on animal implantation studies. These initial studies provide invaluable information on how we can monitor the cells and watch them grow and develop.

Refinement

For the animals welfare we have developed comprehensive post surgical treatment plans e.g for animals receiving a trachea we developed a steam booth to help them breath more easily and remove mucus. Additionally we have approached human clinicians to see how their patients are monitored post implantation and whether we can use similar approaches.

Over the years we have gained significant expertise in monitoring animals receiving and immuno-suppressive drugs and are confident we can give each animal a suitable minimum dose to achieve the avoidance of cell or tissue rejection