Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 21

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

Project Titles and keywords

1. **Intestinal carcinogenesis studies**
    - Cancer, intestine, radiation, x-rays
2. **Troy^{+} stem cells in homeostasis, repair and cancer**
    - Stem Cells, Dynamics, Hierarchy, Tissue regeneration
3. **Understanding liver fibrosis using rat models**
    - Liver disease, scar, rat, therapy
4. **Molecular basis of enteric bacterial infections**
    - Bacteria, infection, diarrhoea, *E.coli*
5. **Cell death, inflammation and cancer in the GI tract**
    - Intestine, stomach, apoptosis, inflammation, cancer
6. **Intestinal barrier function: the role of epithelial and immune cells**
    - Intestine, immune cell, epithelium, microflora, SOCS3
7. **Dietary manipulation or supplementation on rumen function**
    - Rumen, diet, bolus, acidosis, fistula
8. **Understanding inflammation, fibrosis and cancer**
    - Scarring, liver cancer, drugs
9. **Periostin in nerve regeneration and IBD**
    - Inflammation, Inflammatory bowel disease, regeneration, nerves
10. **Adaptations of the host and pathogen during infection**
    - Clostridium difficile, Escherichia coli, inflammation, diet, gut
11. **Gut tumorigenesis**
    - Intestine cancer, polyp, crypt

Project 1	**Intestinal carcinogenesis studies**	
Key Words (max. 5 words)	Cancer, intestine, radiation, x-rays	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	Yes	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 aims of these studies using the genetically altered *Apc*$^{Min/+}$ mouse, an established model of intestinal carcinogenesis, are to investigate the tumorigenic effects of external and internal radiations, a dietary carcinogen and to examine the effects of obesity on intestinal tumour induction. The results will provide information on the relative biological effectiveness (RBE) of ingested radionuclides such as tritium, with external radiation (x-rays or gamma rays). The results from our studies will be published in peer-reviewed international journals, and will thus be publically available.	
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)?	Epidemiological studies have shown alimentary tract cancer to be important site of radiation-induced cancer in humans. The results from these studies will contribute to a better understanding of the risks of radiation exposure of humans and will address important uncertainties in current knowledge of effects at low radiation doses, effects of ingested radionuclides and co-exposures, and inter-individual variation in sensitivity to radiation and will make a	

	valuable contribution to decisions on radiation protection standards.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, inbred and genetically altered. We expect to use approximately 7,000 animals over the 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?	This project requires the use of genetically altered mice and their normal siblings, many of which are needed for breeding purposes only. Mild severity levels will be expected in breeding animals and about 50% of experimental animals; moderate severity levels in the other 50% of animals. The mice will be housed and maintained to the highest accepted UK standards for welfare and will be provided with items to enrich their environment. Some of the mice will bear an intestinal tumour burden and may show moderate signs of this disease, including pale feet, ruffled fur, inactivity and lack of appetite. All mice will be closely monitored for clinical signs of disease, and will be humanely killed at the end of the experiment or as soon as they present with overt signs of disease.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project centres on studies to investigate the effects of ionizing radiation on the gastrointestinal tract. *In vitro* cellular studies have been used to investigate radiation effects in cells but it is not possible to carry out studies of radiation-induced gastrointestinal cancer using cell culture because mammalian gastrointestinal tract structures cannot be reproduced in culture and in mice (as in humans) most of the development and maturation of the gastrointestinal tract occurs after birth. It would not be possible to use lower organisms for these studies, such as invertebrates, plants and micro-organisms (see www.frame.org.uk) because they do not have the same gastrointestinal tract structure as mammals. Mice have been chosen for these studies because they are small mammals that provide a good model

	system for the study of carcinogenesis. The *Apc*$^{Min/+}$ mouse model is an established model of radiation-induced intestinal and mammary carcinogenesis and, as such, it provides the best model for this work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals used will be minimised on the basis of previous experience with this mouse model and with statistical advice using power analysis to determine the minimum group sizes required to produce statistically valid results, aiming for 95% confidence levels. The interpretation of results will also be done with advice from statisticians. Methods applied will include *t*-tests, Poisson statistics and regression analysis, as appropriate to the data obtained. The use of animals specifically for molecular studies will be avoided / minimised by long-term archiving of tumour tissues.
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.	It is not possible to use lower organisms, such as invertebrates, plants and micro-organisms, for these studies as they do not develop cancer or cancers sufficiently similar to those in humans. Mice have been chosen for these studies because they are the lowest vertebrate group in which well-characterized models of intestinal cancer have been developed, they are genetically similar to human and provide a very good model system to study radiation-induced carcinogenesis. Using mice maximises the potential for use of genetic markers in molecular studies and the potential for interpretation of results using genetic databases. All mice will be group housed and maintained to the highest standards for welfare in line with Home Office regulations and approved by our named veterinary surgeon; environmental enrichment is included in their cages. By choosing a well-established model, we minimise unknown effects on mice and subsequent pain, distress and suffering. The signs of the neoplastic disease in our model are well known, and obvious on inspection. Mice carrying the mutation will be monitored daily for signs of the disease.

Project 2	**Troy^{+} stem cells in homeostasis, repair and cancer**	
Key Words (max. 5 words)	Stem Cells, Dynamics, Hierarchy, Tissue regeneration	
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 purpose of this project is to understand the molecular mechanisms by which stomach stem cells maintain adult tissue homeostasis and repair damaged tissues. With Troy being a novel and the only known marker for stomach stem cells this is the first study that addresses their distinct role in stomach homeostasis and in various pathological situations.	
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 is expected to provide novel information about the hierarchy and the dynamics of stomach epithelial tissue. Furthermore, we will evaluate the properties of stem cell proliferation and the signalling pathways that regulate their division during homeostasis, tissue regeneration as well as during tumour initiation. During tumour development resting cells can be activated in an uncontrolled manner. Understanding how this activation takes place during homeostasis and injury response is crucial to improve our knowledge on the basics of cancer initiation and will help us to identify early screening markers that	

	potentially could be used to design specific anti-cancer drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice ~9092 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?	To study the role of stem cells during tissue homeostasis we expect 50% of the animals to experience mild discomfort. This will be related to intraperitoneal injections of inducing agents and will last <1 day. From the studies of tissue regeneration, we expect the adverse effects to be mild. Mainly ~50% of the animals will be experiencing mild discomfort due to the induction of a repair response. As we always aim for the tissue to repair, any discomfort should last no longer than 48h. To induce tumour formation in the mice, we will be using inducible systems. Tumours will only be induced in adulthood in ~10% of the mice. This might result in moderate discomfort to the mice. Animals will be monitored daily and when tumours develop the animals will be humanly killed before the onset of any metastasis. In all cases, animals will be humanly killed after the experiments, either after induction of a tissue repair response or after generation of tumours. We will analyse the presence of particular phenotype by using the molecular, histological or culture techniques available (e.g. appearance of tumours, or tissue repair after tissue injury)
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal studies are unavoidable if we seek comprehensive knowledge and understanding of gene function, physiology and pathology. The necessary animal studies in this Project will exclusively involve mice. In that regard, the knowledge and expertise accumulated from the

	investigation of the mouse is incomparable. To date, there are no alternative methods to fully understand tissue regeneration and tumour initiation in the context of the whole organism. For the majority of the proposed studies, the mouse is the most appropriate animal model because: (i) it is a mammal; (ii) physiology is more extensively characterized in mice than in other mammalian model species; (iii) mice are amenable to transgenic manipulation; (iv) a large number of relevant transgenic and knock out lines are already available; (v) most of our knowledge in tissue regeneration has been obtained from studies in mouse. Nevertheless, this program will make extensive use of the organoid culture (adult stem cell culture) technology, which by itself extensively reduces the animal numbers. The use of this near-native culture system allows this project to comply with the 3Rs (replacement, reduction and refinement) by keeping the mice numbers to be used to minimum.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As mentioned, This program will make extensive use of *in vitro* culture screenings to underscore potential candidate factors influencing cell proliferation and tumour formation, prior to testing these into the animals. Also, as many of the procedures are very well established in the mouse, and don't require additional experiments to test them in this animal model, using mice allows us to reduce the number of animals that would be required if setting up protocols were needed. Finally, whenever possible, we will perform pilot studies with the minimum amount of animals possible, an example would be the validation of new alleles, or combinations of genotypes.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	All studies in this Research Program will involve exclusively inbred mice from different backgrounds. In that regard, the knowledge and expertise accumulated on resident stem cells from the investigation of the mouse is incomparable.

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The experiments will involve creating and analysing transgenic mice and performing stomach regeneration studies following chemically induced cell type specific ablation of the stomach. This can result in transient discomfort for the animals. We will use the lowest doses that will give an effect. When available, the drugs will be given orally, either supplemented on the diet or drinking water, to prevent any stress to the mice. Any animal in distress will immediately be euthanized.

Project 3	**Understanding liver fibrosis using rat models**	
Key Words (max. 5 words)	Liver disease, scar, rat, therapy	
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
		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 has two main aims; Firstly, understand how scars (fibrosis) form in the liver when it is damaged e.g. by drugs, alcohol and/or fat, and to help identify anti-scar drug targets. We then want to test medicines, which have the potential to prevent scar formation. Secondly, ask how protection against developing scaring/fibrosis in the liver when it is damaged can be passed on from either grandfathers or fathers to their offspring. To achieve these aims we will use two experimental systems; Firstly, we will isolate scar-forming cells from the liver and culture them in petri dishes, then perform experiments on these cells to help understand their biology and ask what they do. We will then bathe these cells in different drugs to see if they effect scar cell survival or how much scar they make.	

	The second approach is to induce liver damage and scaring in rats to help understand the biology of the disease and test potential anti-fibrotic (anti-scaring) medicines. We will cause liver damage using chemicals, or by surgically injuring the liver or by feeding rats a modified diet (high fat and high sugar). To see if protection from developing liver disease passed on from father to son affects wound healing and scar formation in other organs we will promote liver injury in dads, then in the sons either surgically injure the kidney or place chemicals into the lung or punch holes in the skin to assess scar formation.
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 outcomes** of our work are; 1. To identify new anti-fibrotic drugs which cause scar cell death, stop them making scars by re-programing their epigenetic code (this is when the DNA or proteins associated with it are chemically modified). 2. Identify biomarkers or imaging tools to help stage the fibrosis or tell us that fibrosis is reversing e.g. if a drug is working. Biomarkers include molecules or proteins released into the blood or changes to the epigenetic code in liver tissue. **Clinical Importance:** Viruses, alcohol, drugs, fat and inherited or autoimmune disease (when the bodies immune system attacks the liver) can cause damage to the liver. When we repeatedly damage our liver scars form, a process termed fibrosis. The scarred liver does not work properly, however if we kill the scar forming cells then the fibrosis melts away and the liver returns to normal. Currently more than 16,000 people die in the UK each year from liver disease and it is the UK 5th biggest killer. Sadly, people who have liver disease die young, with the average age of someone dying from chronic liver disease (CLD) being just 59 years, compared to 83 years for heart & lung disease or stroke. CLD have risen by 40% between 2001-2012 making liver disease the 5th major cause of death. The average age of death from CLD is just 59 years, compared to 83 years for heart & lung disease or

	stroke. There are currently no effective anti-fibrotic drugs to treat CLD and for many patients whose liver disease is getting worse is a liver transplant is the only option. Currently there are no effective anti-scaring drugs to treat liver fibrosis and people with advanced liver disease the only treatment option is a liver transplant. However donor organs are limited and in 2009 only 58% of patients on the waiting list received a transplant. We urgently need to identify new drug targets and test potential anti-fibrotic therapies to address this rapidly growing clinical problem. Our programme of work will help understand how CLD and liver cancer develop and allow us to identify and test new medicines to treat CLD. We also hope to develop imaging methods to help assess disease in living animals.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats, we have estimated using up to 4200 rats over a 5 year project.
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?	All procedures with the exception of protocol 1 are classified as moderate. The liver injury in rats will be caused by either chemicals (carbon tetrachloride), surgical models (blocking of the bile duct) or by feeding of western lifestyle diets e.g. high fat, high sugar diets. The rat will develop liver disease and very rarely show signs of sickness e.g. hunched posture. Very occasionally jaundice (skin becomes a yellow colour) or swelling of the belly may develop in the bile duct surgical model. We will promote disease in other solid organs including; 1. The kidney by surgically tying the ureter, which prevents urine flowing from the kidney to bladder. 2, The lung by putting chemicals into the lung to cause damage. 3. The skin of rats by punching two holes in the skin and watching them heal.

	At the end of the experiment animals will be humanely killed and liver tissue and blood samples will be taken to assess the liver damage and extent of disease. We will use microscopes to look at the scar forming cells, white blood cells and production of scar tissue. We will measure markers of damage in the blood. We will use imaging machines to scan the rats with CLD to help assess the level of the disease in living animals. To see the scars we will give special "tracers" which bind the scar or scar cells. The tracers fluoresce or vibrate when stimulated and can been seen by the special imaging machines. From our current experience we do not anticipate any adverse effects of imaging.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Fibrosis and scarring in an organ is a complex process. The disease develops over many weeks and requires lots of different cells both within the organ and within the blood to talk to each other. In our "inheritance" study we are asking how protection from developing fibrosis can be passed from father to son. To achieve this we need to promote fibrosis in the dad, then breed him with a female rat and then injure their offspring and then compare the amount of scarring to rats bread from injured or un-injured parents. For these reasons these programmes of work can only be conducted in animals and not alternative systems such as growing cells or thin slices of an organ in a petri dish. Wherever possible, we use human tissue to isolate cells or make slices or stable cell line cultures generated from human cells, which we can culture in a petri dish to replace animal models of liver disease. We use stored frozen and wax embedded liver tissue collected from previous studies to help answer our research questions and minimize further use of

	animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies are planned very carefully and we always use the minimum numbers of animals possible to achieve meaningful results. Statistical analysis is used to help predict the number of animals needed to achieve our research aims. Primary cells, cell lines and slices of liver tissue are also used to help reduce numbers of animals used. Before testing drugs in rat models of liver disease we first show that they have a “therapeutic effect” in cultured cells, this way we only test drug that we expect/predict to have a positive response. We use imaging approaches to help reduce group sizes or numbers of time points, which reduces numbers of groups or group sizes.
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 models chosen are the least severe models of liver disease that will allow us to answer our research questions. Using rats to model liver disease and fibrosis allows us to test new drugs, which can be used to treat fibrosis in the lowest vertebrate animal. As rats are closer to humans than mice, it is possible that better dose prediction can be achieved with rats. For example, the chemical model (carbon tetrachloride) is very predictable therefore we know how far advanced the disease will be at a given time point. We have lots of experience running drug studies in this chemical model, therefore we know exactly what time to give drugs and for the length of time to treat the animals. In this model rats receive bi-weekly injections of the chemical into their belly, however before we start the model the rats under a period of handling to minimise the stress of restraint and an injection. We have good surgical techniques and operating theatres/equipment to minimise the risk of infection. In the surgical bile duct ligation model rats receive pain relief and a high level of post-operative care including soaked diet, a warm environment, pain relief and fluids as required to minimize stress and suffering. We work with the vets, pain team and

	animal behaviour/welfare scientists to develop clinical scoring system to help assess the animal's well being and level of disease. Animals are checked regularly and supportive care is readily provided to minimise suffering and improve the rats welfare.

Project 4	**Molecular basis of enteric bacterial infections**	
Key Words (max. 5 words)	Bacteria, infection, diarrhoea, *E.coli*.	
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 this project we focus on two important human pathogens: pathogenic *E. coli* and *Salmonella enterica*. The main aim of the project is to gain better understanding of the infection strategies used by these microorganisms so that effective treatments and prevention strategies could be developed and tested. The project is divided into three interrelated research streams: • Investigation to define pathogenic (virulence) factors implicated in disease • Evaluation of the efficacy of new vaccines • Determination of the health benefits of probiotic bacteria.	
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)?	Infectious diseases are major causes of morbidity and mortality worldwide, especially in developing countries. The emergence of multi drug resistant bacterial pathogens means that some infectious diseases may become untreatable. The studies described in this application will contribute to the improvement of health on several levels. First, we will determine the mechanisms of mucosal bacterial infections. Those mechanisms involve complex interactions between the host and the pathogen as	

	well as a complex immune response mounted by the host to combat the pathogen. By using an in vivo system we benefit from monitoring the immune response throughout the whole infection cycle and are not restricted to a short term infection of a few hours as limited by an in vitro model. This is a prerequisite for the development of effective and rational control measures and for the identification of novel anti-bacterial drugs. Second, we will develop vaccines based on key pathogenic proteins secreted by bacteria and novel bacterial vaccine delivery systems. In this project we will also determine the health benefits of probiotics. Probiotics can be defined as live microorganisms which, when administered in an adequate amount, confer a health benefit to the host. Consumption of probiotic products by adult humans is increasing, although the health benefits are less defined. In this project we will assess the effect of probiotic treatment, either before or during challenge with pathogenic bacteria, on the well being of mice, using the same criteria as those used for evaluation of vaccines.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse – adult. 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?	The mouse models have been well characterised and clinical signs of infection are readily recognisable. Care will be taken to minimise suffering and the human endpoints described will be strictly adhered to. Possible, rare, adverse effects include damage to the trachea, oesophagus, or airways during oral gavage, there is a risk for organ rupture during i.p. injection or weight loss due to the infection. In addition, repetitive anaesthesia can lead to stress and non-recovery. Whole body irradiation can lead to increased susceptibility to infection. We monitor the animals closely and mitigate the adverse effect as much as possible. Experiments will be terminated at the peak of infection or after clearance of the infection. Animals may be killed by exsanguinations under non-recovery anaesthesia, followed by cervical dislocation

	(schedule 1) or by schedule 1.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of animals is an unavoidable consequence of studying the complex physiological processes involved in models of human infection. We study the role of proteins released by gut bacterial pathogens in infection, this involves generation of alterations (mutations) in the gene of interest. While it is essential to obtain full characterization using cultured cells in the laboratory (*in vitro*), the true role of a protein in infection can only be revealed using relevant animal models (*in vivo*). Generally in host pathogen interaction scientists find that in vitro infection data do not reflect the in vivo reality of the infection. Even though in vitro models are primed tools to study cell biology it is not a true model of host pathogen interaction and this is where the differences are. Indeed, others and we have shown that when models of infection strategies developed solely *in vitro* can lead to misleading and unrealistic conclusions. We use a defined set of criteria to assess the role of bacterial protein in infection. When possible and appropriate, we use bioluminescent bacteria that allow us to study colonization dynamics and tissue distribution while reducing significantly the number of animals. Better understanding of how virulence factors function *in vivo* provides a rational for development of specific treatments and vaccine. As far as vaccines are concerned there are no alternative but to test them in animal models as, by definition, their efficacy can only be assessed in the context of a complex immune response found only in animals and testing the effect of probiotic bacteria can only be done using animal models; there are no other alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our laboratory won the 2006 National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) 3Rs prize for developing a natural transmission model of bacterial infection and we have further refined the *C. rodentium* mouse model by developing the use of bioluminescence imaging to monitor infection dynamics noninvasively and in real time**.** We use novel bioluminescent imaging techniques to reduce the number of animals used for testing and adhere closely to the 3Rs

	principles. Pilot experiment will routinely involve 5 animals per group and per time point and will only be carried out when a minimum of two bacterial strains can be tested in parallel with positive and negative control groups. The pilot experiment will be exploited to the best by collecting data for a maximum of scientific parameters. Several mutated bacterial strains are routinely tested in the same experiment so that positive control mice and negative control mice (uninfected) can be shared, thereby reducing the number of animals. All experiments are repeated two to three times (including pilot experiment) to achieve scientific validation and to ensure reproducibility.
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 models described have been well characterised and clinical signs of infection are readily recognisable. After infection, mice are monitored daily for visible signs of illness (cachexia, ruffled fur etc) and weight loss. Care will be taken to minimise suffering and the human endpoints described will be strictly adhered to. The main bacterial infection model we use is self-limiting and the severity is moderate. Experiments will be terminated at the peak of infection or after clearance of the infection. Animals may be killed by exsanguinations under non-recovery anaesthesia followed by cervical dislocation (schedule 1).

Project 5	**Cell death, inflammation and cancer in the GI tract**	
Key Words (max. 5 words)	Intestine, stomach, apoptosis, inflammation, 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
		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 this project is to understand the processes that take place in the gut that lead to or prevent the development of gastrointestinal diseases. Information gained from this project will provide insights into the complex interplay between genetic and environmental influences that regulate how the normal gastrointestinal tract functions, and the mechanisms that may be targeted in order to develop novel therapeutics for inflammatory bowel disease, colon and gastric cancers.	
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)?	Gastric and colon cancer are the 2nd and 7th commonest types of cancer in the UK accounting for 23% of cancer related deaths. Additionally, the incidence of inflammatory bowel disease is rising annually in Western societies and confers an added risk factor for the development of colorectal cancer. The complex mechanisms driving susceptibility to the development of these gastrointestinal diseases will be explored within this project, as understanding these disease processes is vital for the generation of new therapeutic strategies. It is hoped that data generated from this project will ultimately result in the generation of novel drugs that will enhance the quality of life and longevity of inflammatory bowel	

	disease and gastrointestinal cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	The majority of our experiments will be conducted in mice because of the availability of genetically altered strains, however, if necessary a small number of rats may also be used. We anticipate that 8000 mice will be used in experimental procedures 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 majority of mice will be subjected to mild procedures in which a single agent will be administered by injection on one occasion and the mouse will be humanely killed after a short time (1 hour – 24 hours). The doses of substances that will usually be administered will result in structural differences to the inside layer of the gut which will only be detectable with a microscope and will not affect the animal's health. Some studies will require the mice to be administered agents in their drinking water that result in diarrhoea. Similarly to that shown in humans with inflammatory bowel disease, mice will lose weight and some may develop rectal bleeding. It is anticipated that 50% of these mice will experience moderate severity and all mice will be monitored closely throughout the procedure. Some mice will be infected with bacteria that are placed directly into their stomach to change the composition of the normal gastrointestinal microflora. The majority of infection studies will be conducted using *Helicobacter felis*. Mice infected with *H. felis* show no clinical signs during the early stages of infection and short term studies would fall into the mild severity category. However, during longer periods of infection, mouse weight and general condition will be monitored and 10% of these mice are expected to fall within the moderate severity banding. A small number of mice will be subjected to moderate surgical procedures that will either be conducted under terminal anaesthesia for imaging purposes or under anaesthetic with recovery procedures for the implantation of tumour cells in the colon. All animals at the end of every procedure will be killed by a schedule 1 method and tissues will be taken for further analysis.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whilst standard tissue culture techniques are informative, they are not able to recapitulate the complex environment found in the gastrointestinal tract. The interactions of multiple cell populations such as epithelial cells that form the inside layer of the gut, with cells of the immune system are needed to address all of our research questions, and interactions such as this are currently unable to be accurately generated in cell culture experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible, we will use tissue culture techniques and human tissue biopsies to address our research questions. When animal experiments are necessary, group sizes will be determined using power analysis. The significance level will generally be set at 5% and the power 80%. The group sizes required for each experiment will depend on the coefficient of variation. We have previously established that a minimum of 4 mice per experimental group are required for reliable data generation in radiation and mucositis studies, 6 mice are required per group for *Helicobacter* infection studies and that there is a greater degree of variation during experimental colitis studies resulting in the requirement for minimum group sizes of 10 mice. When necessary, we will seek statistical advice about 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.	The structure and function of the gastrointestinal tract of mice is similar to that of the human and there are many genetically manipulated strains of mice are available that are suitable for the proposed studies. Therefore, the majority of our studies will be conducted in mice. Data from our previous and future experiments will be used to determine the minimum doses of substances required to exert biological effects in order to avoid doses that induce significant toxicity. Where possible, time-points will be analysed when there are early signs of disease but no clinical ill health. Zebra fish and drosophila are being used increasingly for the study of biological systems and these organisms are both suitable for genetic manipulation. The structure of drosophila intestine is however substantially different from that of mammals and would be unsuitable for most of our studies; however, the zebra fish intestine does display structural similarity. Little is currently known about the dynamics of zebra fish intestine, however we will

	monitor the field of investigation closely and will include zebra fish in our experimental plans when information about the intestinal epithelia becomes sufficient to enable us to design appropriate experiments.

Project 6	**Intestinal barrier function: the role of epithelial and immune cells**	
Key Words (max. 5 words)	Intestine, immune cell, epithelium, microflora, SOCS3	
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
	✓	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 aims of this project are to understand genetic and environmental risk factors for chronic intestinal 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)?	Prevention and treatment of chronic diseases such as colorectal cancer, inflammatory bowel disease and allergies. The causes of these diseases remain poorly understood, and consequently, there are currently limited effective prevention or treatment regimes	
What species and approximate numbers of animals do you expect to use over what period of time?	This work will use mice only. Over the 5 year period of this project it is estimated that the work will use less than 2000 mice. The transgenic mouse breeding strategy aims to generate as few 'unusable' mice as possible. Approximately 20% of the mice used (about 200) may be purchased/transferred from approved suppliers and/or licensed institutions.	
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	Animals will be fed various bacterial, chemical and nutritional supplements to alter their intestine replenishment and repair. Chemical and bacterial agents will also be used to promote colonic tumour development. Animals will be monitored daily, weighed, and will be humanely sacrificed prior to any chance of developing occlusive lesions, haemorrhage	

end?	or any other side effects associated with late-stage tumour growth or chronic inflammation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To be physiologically relevant, the study of host:microbe interactions requires the use of animal models, as currently in vitro intestinal models are insufficient to replicate certain critical functions, such as how the intestine responds to infection and how cancer cells evade the immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experiments and methods of analysis of the results have been considered when calculating the numbers of animals required and these will be discussed with an independent statistician.
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 chosen because it is a mammalian model and transgenic animals specific to the gene of interest are available. The models of intestinal replenishment and repair proposed are widely-recognised and our group has previous experience of using the majority of proposed procedures. Pilot studies to find the most effective dose at which no adverse side-effects are observed will be performed. Schedule one cull will be performed any point during the procedure if there is any indication of ill health.

Project 7	**Dietary manipulation or supplementation on rumen function**	
Key Words (max. 5 words)	Rumen diet bolus acidosis fistula	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	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 objectives of this project are to investigate the effects of diet on rumen function, and to develop improved efficacy of nutritional supplements and rumen boluses to combat a host of production diseases that are common across the globe. Improved rumen function will lead to significant improvements in animal productivity, health and welfare.	
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)?	Production animals, in particular cattle and sheep, will benefit from this project as new feeding strategies, or nutritional supplements/boluses are developed. These benefits will be related to productivity, health and welfare.	
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 119 cattle and 516 sheep may be used over this 5 year project.	
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?	Similar studies have been conducted in previous studies and adverse effects have shown to be minimal. Rumen cannulations, the surgical process whereby a permanent access point is made through the flank of the animal to facilitate access to the ruminal part of the gastrointestinal tract, are likely to lead to moderate pain and discomfort, but this has been shown previously to be only transient. All other	

	studies are classified as mild. At the end of this project some of the animals will be euthanased, while others will be discharged from ASPA and 'returned' to farm stock due to the negligible effects of the studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of live animals is necessary as it is not possible to model in vitro the complex nature of rumen function, rumen contractions, feed interactions, microbiota and effects of the production cycle.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be designed to use the minimum number of animals required to establish statistically significant outcomes. Typically 6, but occasionally more, animals will be utilised in each experimental study per group to determine response to a specific nutrient. This number of animals is the minimum required to determine nutrient levels, taking into account the biological variation in uptake expected as a result of past experience. Number of animals will also be reduced via continued use for subsequent rumen function studies on this project licence, since previous studies have demonstrated no adverse effects to date.
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 rumen-cannulated cattle and sheep has been well validated in the literature. Cattle and sheep with cannulae generally lead a longer life of relative luxury compared with most commercial livestock. In order to prevent unwanted pregnancies female cattle and sheep will be managed separate from entire male animals on the farm. Interventions are generally restricted to brief confinement for placement or removal of the subject bolus or bag or collection of rumen fluid and/or blood and faeces. It would not be possible to do these studies on dead/slaughtered animals given that we will be monitoring changes in pH, microbiota and rumen function. Animal suffering will be minimised by using experienced licensees for all procedures. In addition, feed products, supplements or boluses are produced under strict regulatory frameworks and are not anticipated to have any adverse effects.

Project 8	**Understanding inflammation, fibrosis and cancer**	
Key Words (max. 5 words)	Scarring, liver cancer, drugs.	
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)	Fibrosis is characterised as the build up of scar tissue in a damaged organ and its gradual accumulation correlates with a steady decline in organ function. Fibrosis can affect many organs and some of the events controlling the development of fibrosis that are common in different organs regardless of the type of injury to the organ. It is thought that fibrotic disease follows a common course; repeated organ damage → persistent inflammation → fibrosis → increased risk of developing cancer. This is known as the "inflammation-fibrosis-cancer axis". Additionally the cells that make scar tissue in different organs are similar; therefore a drug that prevents fibrosis in the liver could also prevent fibrosis in the lung, skin or heart. Currently there are no drug treatments for fibrosis in any organ and the only therapy for liver cancer only extends life by 3 months. **OVERARCHING AIM:** Use a combination of physiological, biochemical, histological and molecular approaches to identify proteins and signalling pathways that influence every point of the inflammation-fibrosis-cancer axis. Our research plan is to use a multi-organ approach to understand the	

	"core" molecular events underpinning these diseases to discover new targets and test new drugs to treat fibrosis. Objective1: understand how multi-organ fibrosis develops and identify potential drug targets and therapies to prevent these diseases. Objective2: understand how liver cancer develops. **Scientific Aims/Key elements:** 1. To understand the biology of wound healing and fibrosis in the liver, skin, knee/joint, heart or lung. 2. Understand the molecular and cellular mechanisms promoting liver cancer. 3. Understand how epigenetic changes that occur as a consequence of CLD/fibrosis can be transmitted to future generations to protect offspring from developing CLD and fibrosis. 4.Test therapeutic agents that target signalling pathways or molecules, or modify lifestyle (e.g. diet and/or exercise) contributing to the inflammation-fibrosis-cancer axis. 5.Develop a rodent model of arthrofibrosis and test potential therapeutic compounds. 6.As part of the overall program of work, to develop and refine imaging techniques to monitor fibrosis and liver 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)?	**The primary outcomes of our work are;** 1. To identify new drugs that prevents fibrosis in different organs. 2. Identify biomarkers or imaging tools to help understand/study the progression of fibrosis or tell us that fibrosis is reversing e.g. if a drug is working. Biomarkers include molecules or proteins released into the blood or changes to the epigenetic code in liver tissue. 3. Develop new imaging methods to detect/visualise fibrosis in organs. **Clinical Importance:** Organ fibrosis is a debilitating disease and as scars accumulate the ability of the organ to do its job declines. It's estimated that fibrosis may cause up to 45% of deaths in the western world. Treatments for fibrotic diseases are limited because there is a lack of effective drugs. The number of

	people with fibrosis is rapidly increasing; therefore managing this disease epidemic is creating significant social, economic and healthcare burdens. Our programme of work will help us understand how inflammation (redness and swelling caused by white blood cells) and fibrosis (scarring) occurs in an organ to identify and test new medicines to treat these diseases. We want to understand how liver cancer develops and test new drugs, which prevent cancer growth. We also hope to develop new imaging methods to help assess liver disease in living animals.
What species and approximate numbers of animals do you expect to use over what period of time?	Adult mice and rats. We estimate that over 5 years we will use up to 30,000 mice for maintenance and breeding of different mouse lines. Experimental procedures are estimated to be up to 28,900 mice and up to 600 rats over 5 years. The animals will be either generated in-house or purchased from an authorised supplier.
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?	All procedures are classified as moderate except acute carbon tetrachloride (chemical) liver injury (mild), cell isolation from liver tissue (non-recovery), and protocols surgically tying off the bile duct & liver failure models e.g. paracetamol overdose (severe). At the end of all studies blood samples and organs will be taken to analyse the extent of disease and determine if therapies have been successful. Mice will be given chemicals (carbon tetrachloride or thioacetamide) or surgically injured (tying off the bile duct) or fed a western lifestyle diet e.g. high fat, high sugar diets to cause scarring in the liver. ***Adverse effects:*** Mice will develop liver disease and very rarely show signs of sickness e.g. hunched posture. Bile duct ligated (BDL) mice, where we surgically restrict the bile duct, occasionally become jaundice (skin becomes a yellow colour) or get swelling of the belly. In the dietary models most mice will gain weight, but in the methionine and choline deficient (MCD) diet model we expect mice to loose weight. Liver growth is assessed by surgically removing up to 70% of the liver. There are no adverse effects in the liver growth model other than potential surgical

	complications; bleeding or inadequate anaesthesia. Liver cancer: we give mice a chemical that causes liver cancer to develop over a period of up to 60 weeks. ***Adverse effects:*** Mice will develop liver cancer but this will not result in liver failure. Liver cell death will be promoted by giving mice chemicals or drugs at toxic doses (e.g. paracetamol). ***Adverse effects:*** The liver injury caused by the drug/compound will be either lethal where mice are humanely killed as the liver fails or sub-lethal where mice will recover. Lung inflammation/scarring will be induced by putting chemicals or allergens in to the lung to cause damage. ***Adverse effects:*** Mice will develop lung disease and can loose weight during the first week of the models but regain weight after this time. Skin inflammation/scarring will be induced in the mice by either giving agents, which irritate the skin or chemicals that cause scarring. Punching two small holes in the skin and then watching them heal will be used to study how skin wounds heal. ***Adverse effects:*** The skin may become red (inflammation) and the skin will become thicker. The wounds created by “skin punching” are superficial and therefore do not bleed. Cardiac fibrosis will be induced by increasing blood pressure, which stresses the heart by chemical (angiotensin) infusion or surgically restricting the aorta. We may directly injure the heart by freezing a small area of the heart; once damaged cells are lost they are replaced with scar tissue. ***Adverse effects:*** The heart will become fibrotic, but the extent of the injury is not sufficient to cause a heart problem. Knee fibrosis will be induced by surgically removing/partially removing the fat pad in the knee joint. ***Adverse effects:*** The knee may be stiff and the joint movement slightly restricted. Bone marrow chimera: normal or genetically modified (GM) mice will be irradiated to remove the white blood cells and then given a new immune cells from a donor mouse of a different background e.g. normal in to GM. ***Adverse effects:*** Mice may be sick and loose weight after irradiation but will recover once the immune system has been replaced.

	At the end of the experiment animals will be humanely killed, the affected organ (liver, lung, skin, heart or knee) will be removed and blood samples will be taken to assess the extent of disease. We will use microscopes to look at scar cells, white blood cells, dividing or dying cells and scar production. We will measure damage markers in the blood.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Organ fibrosis or development of liver cancer is a complex process. These diseases develop over many weeks and require different cells both within the organ and within the blood to talk to each other. In our “inheritance” study where we are asking how protection from developing fibrosis can be passed from father to son. To achieve this we need to promote fibrosis in the dad, then breed him with a female mouse and then induce liver fibrosis in their offspring and then compare the amount of fibrosis to mice breed from fibrotic or healthy parents. Therefore these programmes of work can only be conducted in animals and not alternative systems such as growing cells or organ slices in a petri dish. Wherever possible, we use human tissue to isolate cells or make slices, which we can culture in a petri dish to replace animal models of fibrotic disease. We use stored frozen and wax embedded liver tissue collected from previous studies to help answer our research questions and minimize further use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies are planned very carefully. We always use the minimum numbers of animals possible to achieve meaningful results. Statistical analysis is used to help predict the number of animals needed to achieve our research aims. We use imaging approaches to help reduce groups sizes or numbers of time points which reduces numbers of groups.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	All of the disease models described in the plan of work are the least severe models that will allow us to answer our research questions and achieve our aim of further understanding how inflammation, scaring (fibrosis) develops in multiple organs. This will help us identify new drug targets and test potential drugs,

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	which inhibit these targets to develop new anti-inflammatory and anti-fibrotic medicine. We have good surgical techniques and mice or rats used in surgical models will receive pain relief and a high level of post-operative care to minimize stress and suffering. We will use methods to watch the mice and perform behavioural tests to help refine our procedures. We will monitor all of the animals carefully, and apply well-defined humane endpoints to limit the severity of the disease processes. We will provide supportive care (for example fluids and more palatable food) to reduce the effects of the disease process. All models will be performed in mice except surgically inducing fibrosis in the knee, which will be performed in rats. This is because disease processes are complex and involve lots of different cell types, therefore to understand the disease process we need to use whole animals. Mice are used because the disease models have been very well characterised in this species. In addition, we can genetically modify mice to help understand the role of specific factors involved in the disease process. Rats are use for the knee fibrosis because their joints are larger which is more amenable for surgery and allows the disease to be assessed more accurately and offers a better system to test new treatments.

Project 19	**Periostin in nerve regeneration and IBD**	
Key Words (max. 5 words)	Inflammation, Inflammatory bowel disease, regeneration, nerves	
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)	We want to understand the role of Periostin in the regeneration of tissue after injury, and understand its significance in the development and progression of inflammatory 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)?	In the first instance our work will expand the knowledge we have on nerve regeneration and Inflammatory bowel disease. Regeneration of nerves after injury does occur, but only to a certain extent, which leaves the patient with disabilities. Finding molecules that enhance regeneration of nerves will be beneficial to any person having an accident that impairs their nervous system. Additionally, these analyses will give us more insight into the development and degeneration of the nervous system and therefore can potentially also be helpful for people with nerve degeneration related illnesses, such as Multiple sclerosis and Parkinson. IBD is an illness effecting I in 250 people leading to colorectal cancer in long-term patients. We want to	

	analyse mechanisms leading to the development and progression of this illness and hopefully our findings can contribute to the development of new and improved treatments for this condition.
What species and approximate numbers of animals do you expect to use over what period of time?	Genetically modified and wild type control mice. 300 mice for the nerve regeneration and 300 mice for the IBD experiments over the period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Regeneration: (Moderate) The animals will be operated under anaesthesia. Post operatively the animals are able to walk and climb with only minor impairments. They eat and drink normally and experience very limited if any discomfort. IBD: (Severe) The animals will be treated with substances causing inflammation of the bowel. During this time the animals will become unwell and might suffer from diarrhoea. We will examine both their behaviour and weight as well the carrying out endoscopy on the intestine to judge how well the different animals can cope with the inflicted illness. Should the animal should be in too much pain, it will be culled. We have animal carers, who are highly experienced in animal behaviour and will also monitor the animals for signs of pain.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Both regeneration and inflammation are highly complex processes that cannot be recapitulated in cell culture or invertebrates.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our collaborators and we are experienced in these procedures and therefore can generate reproducible results. This in turn means that we only have to use a minimum number of animals to get statistically relevant results. Further our studies allow following single animals over a time course.
3. Refinement Explain the choice of species	Our experiments have to be done in mammals in order for the results to be applicable to humans (our end goal is to find treatment for human illnesses). The

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.	mouse is the best choice in that respect that it has been used in animal experiments for a long time and all the treatments involved have been checked and optimized for this species. Moreover we are using genetically modified mice (i.e. Periostin knockout). This entails that mice are the species of choice. We know the animals very well and can easily spot any signs of discomfort.

Project 10	**Adaptations of the host and pathogen during infection**	
Key Words (max. 5 words)	Clostridium difficile, Escherichia coli, inflammation, diet, 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
		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)	To understand the way in the gut bacteria *Clostridiun difficile* and *Escherichia coli* cause damage to the gut of the host which can result in chronic and relapsing disease. This will be achieved through either mutating the bacteria to prevent it making specific factors associated with the disease process, or, by modifying the commensal organisms that flourish in the environment of the gut through manipulation of diet or use of drugs. It is expected that this information will provide new insights into how best to reduce or eliminate these disease associated organisms through manipulation of the host's own defences.	
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)?	Both of the organisms described cause significant levels of disease and suffering in both humans and animals. Greater understanding of the manner in which these bacteria cause infection process will provide new targets for intervention. In addition, as bowel inflammation is becoming an increasing problem in the western world, an understanding of the interaction between host immunity, diet and the opportunity for bacteria to establish infection may serve to improve health more generally.	
What species and approximate numbers of animals do you expect to use over what period of time?	Over a 5 year period, the project would be expected to use a maximum of 2000 mice and 600 hamsters.	

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?	Adverse events will be dependent upon the model used. In the majority of infections, animals would be expected to experience minimal suffering. However, to ensure the welfare of animals, all will be subject the appropriate monitoring. For all of the infections, specific and defined endpoints have been identified and are described that will limit animal suffering. For hamsters in which infection is acute and death rapid, telemetry has been employed to allow the use of body temperature as a defined end point and limit suffering. In the case of all protocols, there are defined limits to which animals can remain on procedure before they are culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Increasingly, knowledge of the role of members of the microflora of the gut is showing that these organisms play an important role in maintaining the health and integrity of the gut. As we are unable to culture these organisms, it is essential that we have the opportunity to study the role they play in protection against infections in the context of a live animal. Whilst limited study of individual steps in the infection process can be studied *in vitro* (and will be undertaken as a first approach where possible), full understanding of the initiation and maintenance of disease is only achievable when multiple factors including host immunity is considered.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Group sample size will constantly be evaluated and updated to ensure that the appropriate and statistically relevant numbers of animals are used within each experiment. In situations in which new mutants/methodologies are tested, work will initially be performed using small numbers of animals. Data from these experiments will allow calculation of appropriate sample size.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the lowest vertebrate group in which the biological interaction between these two pathogens and the host can be determined.

Project 11	**Gut tumourigenesis**
Key Words (max. 5 words)	Intestine, cancer, polyp, crypt
Expected duration of the project (yrs)	5 years
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X Basic research Translational and applied research Regulatory use and routine production Protection of the natural environment in the interests of the health or welfare of humans or animals Preservation of species Higher education or training Forensic enquiries Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We use microscopy and cell biological assays to study the physiology of the large and small intestines. We are particularly interested in the differences in cellular physiology of the gut in pre-cancerous states associated with specific mutated genes in animals, as these may be good representations of similar states in humans.
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)?	By observation of differences in physiology between normal and cancer-susceptible gut tissue, we hope to identify the first steps of cancer development (tumourigenesis) and how interactions between different genes modify this susceptibility. Studies of early tumourigenesis in other systems have historically identified new drug targets and prognostic indicators for cancer patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Our plan is to breed transgenic mice that express useful fluorescence markers in gut cells and/or carry mutations in genes known or suspected of involvement in the development of colorectal cancer. We expect to use up to 10000 animals in maintaining our breeding colonies and for providing tissues for analysis. Up to 1000 of these might receive further interventions

	during their lives.
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?	Available mouse lines that express fluorescent proteins experience no detrimental phenotype. Our primary colorectal cancer model mouse will carry a mutation in the Adenomatous Polyposis Coli (APC) tumour suppressor gene. APC mutants can develop multiple pre-cancerous polyps in their gut but, since we are interested in the very early stages of tumourigenesis, the animals will be killed humanely before any clinical signs of disease are observed. We will occasionally irradiate mice to spur cell proliferation, and add cell markers in order to trace individual cellular lineages and determine their specific role in tumourigenesis. Both of these treatments could, in the long term, be tumourigenic in their own right, but in our experiments will be applied only a few weeks before the animals are killed humanely for tissue analysis. There will therefore be little risk of this harm actually being caused. The animals will be killed humanely and then the gut tissues removed for immediate analysis or for disaggregation and culture of the cells in the laboratory. In the microscope, we will trace the cellular behaviour in the context of their native tissue environment and quantify any differences between wild type and cancer-susceptible mutants.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Previous studies have involved experiments in established cell cultures, which cannot take into account the effects of the native cellular environment. Other previous studies have used fixed and stained tissue. These only allow snapshots in time. We need fresh tissue to observe processes such as cell division, migration, and onset of tumourigenesis. We chose the mouse because well-characterized models of human colorectal cancer already exist in this species.
2. Reduction	We have experience in keeping gut tissue alive ex vivo for up to a week of continuous

Explain how you will assure the use of minimum numbers of animals	experimentation. New mouse tissue, therefore, needs to be harvested relatively infrequently. Further, since the intestine and colon are very large organs relative to the amount of tissue that is needed for a single experiment, many independent experiments will be done in parallel from the gut of a single mouse.
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 course of disease in ApCM1th/+ mice is well- characterised and all animals will be killed humanely before the point at which clinical signs might be expected.