Annex A

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2017

Volume 1 (granted between 1st January to 30th June)

PROJECT 1

NON-TECHNICAL SUMMARY (NTS)

Project Title	Regulatory Aquatic Ecotoxicology
Key Words	Aquatic, Ecotoxicology, Fish, Regulatory
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

To determine acute and chronic effects of test substances (e.g. chemicals, formulations, pharmaceuticals, discharges etc.) to fish for regulatory purposes.

The data generated from these tests as well as algae and invertebrate toxicity data will be used as evidence submitted to regulators for environmental risk assessments of the test substance tested.

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)?

To ensure the environment is protected from any adverse effects of the test substances we have gathered data from. The regulatory authorities will use the data generated to ensure the test substances are safe for use and apply restrictions to manufacture, use, transport and disposal to mitigate risk. The data are used to ensure the test substance is appropriately labelled with risk phrases and packaging.

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

The main test species used are below, however other fish species may be used if required. Rainbow trout (Oncorhynchus mykiss) Fathead minnow (Pimephales promelas) Sheepshead Minnow (Cyprinodon variegatus) Japanese Medaka (Oryzias latipes) Zebrafish (Danio rerio) Common carp (Cyprinus carpio) This project license will use up to 36000 fish in the five-year period.

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

The fish are tested for acute and chronic effects which are likely/expected to cause mortality and other sub lethal effects. The fish will be exposed to single concentrations as well as a range thus the effects will not be the same of all organisms tested. Levels of severity will be moderate or severe. Fish will be humanely killed at completion of the test or at a point where by they are deemed moribund if appropriate.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Regulatory authorities require fish testing to understand the effects the test substance may have on the environment. The recognised regulatory guidelines which will be followed using fish species as a data requirement for the submission of approvals of test substances.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Numbers of fish will be reduced where ever possible to obtain a statistically acceptable result for the client and the regulator.

The guidelines followed give numbers of fish to use, this should be followed and minimum numbers used to obtain statistically valid and robust results.

Limit tests (Single concentration) will be used where possible to reduce the need of a multiple concentrations.

Refinement

Explain the choice of animals 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.

Refinement

The use of range finding tests and other data generated from other testing will be utilised to ensure the range or limit chosen will fulfil the regulatory requirements and the success of the test.

This project will follow or base the methodology of the studies on recognised international guidelines (e.g. OECD, OPPTS).

If moribund fish (fish showing no response to external stimuli and/or little respiratory activity) are found they will be removed and humanely killed.

PROJECT 2

NON-TECHNICAL SUMMARY (NTS)

Project Title	Determining efficacy of novel cancer therapies
Key Words	Oncology, Tumour, Efficacy, Pharmacodynamic
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Whilst current cancer treatments provide some survival benefits (50% survive cancer for 10 years or more in 2010-2011), they are often associated with significant side effects. Thus there is a clear need for improved and better tolerated medicines that can be used either alone or in combination with existing or other new therapies.

The aim of this project licence is to develop therapies that reduce, inhibit or prevent the growth of tumours leading to new and improved cancer therapies.

Specifically the licence will be used to:

1. Investigate exposure and tolerability of new drugs and/or combinations
2. Develop new tumour models to test novel mechanisms
3. Profile novel therapeutic agents and/or combinations in tumour bearing models to investigate efficacy, development of resistance and support design of clinical dose and schedule.
4. Investigate target engagement of novel therapeutic agents ex vivo or in vivo in naïve animals
5. Investigate efficacy in inflammatory models to support novel immuno oncology targets
6. Profile novel therapeutic agents and/or combinations in tumour models where tumour develops within target organ

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 using targeted therapy approaches, the treatments should be more effective and should have significantly reduced side effects than those associated with current therapies. This will significantly improve the cancer patient’s quality of life and overall survival.

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

Only mice will be used on this project. Up to 58000 mice will be used over 5 years.

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

Pilot studies will be performed to confirm the tumour types and compound doses are tolerated. In these studies we may observe weight loss and/or changes in clinical signs, such as social isolation or ruffled fur. Animals will be closely monitored and

humanely culled if adverse effects are observed. Animals are monitored by trained staff, with referral to the Named Animal Care and Welfare Officer, veterinary staff and Project Licence Holder as necessary. Depending on how the compound works, target engagement may be assessed in non-tumour bearing models. These models may require activation of an immune response and as such there may be transient signs of inflammation, including changes in behaviour. If these signs persist for longer than 4 hours then animals will be humanely culled. The majority of mice used on this licence will be used in tumour models where tumour grown superficially. Adverse effects related to tumour inoculation include brief discomfort or pain, but this will be minimised by application of good technique. The tumour types used are well tolerated and only two superficial tumour will be used per animal. Tumour size and condition is monitored closely on a daily basis. In some cases tumours will be implanted in organ of interest as this is more clinically relevant. We may observe weight loss and/or clinical signs as a result of tumour growth, but these will be minimised by applying early endpoints from pilot studies. The least invasive tumour site/line and earliest endpoints to achieve the scientific aims will be used. Animals will be culled if the tumour results in significant pain or distress. In these studies clinical signs related to the compound may be seen and mild to moderate signs of toxicity are possible. Animals will be humanely killed if this persists. All animals will be regularly monitored for weight loss and general condition. Weight loss as a result of repeat anaesthesia may occur and this will be minimised by correct dosing and good maintenance of body temperature. Animals where the immune-system is compromised will be housed in sterile conditions. The protocols are classified as moderate severity. Animals will be humanely culled at the end of the study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Non-animal alternatives are used in the identification and selection of compounds and generally include measurements of the likely effect of the agent on the target cells. Activity in particular cell types however, cannot predict the likely *in vivo* activity given the complexity of issues such as bioavailability, metabolism and elaborate physiological interactions associated with tumourgenesis and therefore the whole animal is needed for the studies proposed in this licence.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To maximise the scientific integrity of data generated and use the minimum number of animals, an in house statistician will review all experimental design and analyses. Where applicable the following statistical guidelines will be used:

1. Statistical test defined in advance and optimised to ensure least possible animals are used that will result in meaningful results
2. Definition of meaningful biological change and measurable endpoints.
3. Estimates of biological variability used in sample size and power calculations.
4. Animals allocated in an optimal way based on estimates of biological variability established from historical data, pilot studies or published data.
5. Regular monitoring and updating of biological databases with regular review of group sizes.
   An experimental protocol is written for each experiment including:

- a statement of the objective(s)
- a description of the experiment, detailing experimental treatments, the size of the experiment (number of groups, number of animals per group), duration of experiment, scientific endpoint

Refinement

Explain the choice of animals 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.

Refinement

Only mice, including transgenic and immune-deficient strains, are used on this licence. Using non-mammalian species of lower neurophysiological sensitivity is not possible since they lack the appropriate tissue physiology. Therefore they cannot be used to predict exposure and efficacy in humans.

Within this project specific mouse strains or natural mutants are used e.g. nude mice. Genetically altered animals may be used in order to achieve the scientific objective. The most appropriate strain of mice will be chosen based on previous in-house or external data for each model / project. For human tumour lines immune-deficient animals are required to support the growth of the tumour, the least immune-deficient strain required to promote good, reproducible tumour growth will be used. The optimal conditions for tumour growth will be developed in pilot growth curve studies. Pilot studies to confirm tolerability will be performed on all compounds and combinations prior to progressing studies into the large anti-tumour efficacy studies.

The use of microsampling where possible has refined the process of collecting blood. Where appropriate, to reduce the number of animals used, multiple tissue samples for PD analysis will be taken from each animal.

PROJECT 3

NON-TECHNICAL SUMMARY (NTS)

Project Title	Nutrition of growing & mature ruminant livestock
Key Words	Dairy cows, Sheep, Nutrition, Efficiency, Environment
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The aim of this programme is to increase our understanding of the mechanisms that contribute to the inefficient use of feeds by cattle and sheep, and therefore affect the output of pollutants such as nitrates, ammonia, and methane. For example, dairy cows use only approximately 25% of the feed protein they consume to produce milk protein. Ruminant livestock, and dairy cows in particular, contribute significantly to the UK agriculture's emissions of greenhouse gases and other pollutants. Livestock contribute approximately 50% of UK's methane emissions, about 85% of which comes from enteric fermentation, the remainder coming from manures. At the same time, there is a need to maintain the home-grown production of dairy foods in the UK, to support the agricultural industry and reduce the reliance on imported foods.

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

This project will allow an improved understanding of the interactions between feed and rumen microbes (colonisation, degradation, fermentation, and microbial protein synthesis) that will enable feeding to improve the efficiency of diet utilisation. This will improve the output of ruminant products (meat and milk) and help reduce the environmental impact of ruminant agriculture. For example, an improvement in the use of feed protein for meat and milk production will reduce the amount that is excreted, thereby reducing nitrous oxide (a greenhouse gas) and ammonia release into the atmosphere.

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

Cattle – approximately 400 over 5 years Sheep – approximately 400 over 5 years

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

A small number of animals, approximately 20, will be fistulated at the rumen (cattle and sheep) and/or small intestine (cattle only), and the surgery for this is a moderate level of severity. Following surgery, these, and other animals used by the project, will be subjected to regulated procedures such as restraining them in stalls to measure feed intake, taking blood samples, and collecting outputs of faeces and urine using harnesses and chutes. Some animals will be used in short-term research that may last for between 2 to 4 months, whereas other animals will be monitored for years as part of their normal growth and development. These procedures carry a mild level of severity. At the end of the procedures, those animals that have not been surgically modified will be re-homed in the establishment's herd or flock, or will be sent to

slaughter as part of the normal supply chain for human consumption. Those animals that have been surgically modified are a valuable resource, and if their general health and well-being is good they will be transferred to another project licence. Cannulated animals often live for longer than a cow or sheep on a commercial farm, and will be euthanised when their general health and well-being starts to deteriorate, for example with the onset of arthritis.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

For some of the work, measurement of growth or milk production of animals fed particular diets is required. In addition, whole-body utilisation and partitioning of nutrients between productive and non-productive (pollutant) outputs may be measured. These data cannot be collected using *in vitro* techniques, and therefore animals must be used for those parts of the work.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

All work carried out will follow protocols of work that ensure the maximum amount of information is obtained from the minimum resources required to be statistically valid. Use of changeover design experiments, where appropriate, efficiently controls random variation and therefore fewer animals can be used. Where changeover designs cannot be used (e.g. growth studies) more animals may be required and/or the technical constraints of the experimental design have to be accepted. Some work combines *in vitro* and *in vivo* measurements, e.g., initial screening done using lab-based models of the rumen, followed by field trials of the most promising treatments.

Refinement

Explain the choice of animals 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.

Refinement

Nutrition studies of farm livestock need to be applicable to farming conditions, and therefore work investigating milk or meat (growth) production in cattle and sheep requires the use of those animals housed under normal commercial practises. Similarly, the highest standards of animal health and welfare must be maintained throughout any experiment in order for results of the work recognised by other scientists and farmers to be transferable to productive farms.

The welfare of the cannulated animals used in this work will be safeguarded using aseptic techniques and refined methods of anaesthesia during surgical operations. Following surgeries, animals will be monitored closely and given pain-killers during their recovery. Cannulated animals will be washed daily to ensure they are kept clean, their skin is not irritated by leaks of digesta, and flies are not attracted to the cannulae.

PROJECT 4

NON-TECHNICAL SUMMARY (NTS)

Project Title	The study of epithelial cancer genes
Key Words	Breast, Lung, Cancer, Targeted therapy
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

We aim to identify the genes responsible for the development of different types of breast cancer and lung cancer.

In particular, the research will focus on an aggressive form of breast cancer which has a high possibility of spreading to other tissues, and patients with this cancer have low rates of survival. Identifying genes that are responsible for the development of breast cancer is essential for the development of treatments in the future.

We will also be studying lung cancer. Nearly 45,000 new cases of lung cancer are diagnosed annually and nearly 35,000 people die from the disease in the UK every year. Broadly speaking there are two major types of lung cancer - small cell lung cancer (12% of cases) and non-small cell lung cancer (NSCLC) (88% of cases). There is an urgent need for the development of more effective treatments for NSCLC as currently only 16% of patients survive for 5 years or more after their initial diagnoses. To improve that, a better understanding of how the various types of lung cancer develop is required.

We will carry out genetic analysis to identify and characterise key factors that drive the development of breast and lung cancers in the hope that, in the future, this knowledge may help in the development of new treatments.

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

Results from this project could have an impact on the 100,000 breast and lung cancer patients diagnosed every year in the UK. Our study aims to: 1) Increase the understanding of how types of breast and lung cancers develop; 2) Identify new biological ‘markers’ to enable better cancer diagnosis.

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

Mouse – 42,000 over 5 years. Out of this number, 20,000 mice will be used for breeding and maintenance of genetically altered animals. 22,000 will be used for breast and lung cancer studies.

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

We expect the majority of the animals will have mild adverse effects from our work, indeed 20,000 mice will be used for breeding and maintenance of genetically altered animals. For some animals, a moderate severity limit is expected, particularly if the

animals develop tumours. However, we have protocols and humane end-points in place to minimise suffering in these animals, and will not allow any animal to suffer more than a moderate level of pain, suffering, distress or lasting harm. Animals expected to develop tumours will be constantly and carefully monitored for any signs of ill health or distress, and no tumour will be allowed to grow beyond 1.2cm2. We expect all tumour bearing mice to exhibit no more than moderate severity levels of distress.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Cancer is a complex disease that develops in intact tissues. It is necessary to have a realistic model, which is amenable to genetic, and biochemical studies whilst maintaining the tissue architecture. The mouse allows us to perform detailed genetic and biochemical studies whilst maintaining the 3D organisation and normal physiological environment of tumours in the body. We will aim to use human cell lines in culture dishes in the laboratory, whenever possible, to perform some of our studies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will restrict our analysis of genes using mice to those showing potential clinical relevance in large patient datasets.

1) We will use the latest gene editing technology (called CRISPR/Cas9) to perform our experiments which will allow us to reduce the number of animals needed to generate a genetically modified mouse.

2) When possible we will perform pilot studies on human cell lines in the laboratory before moving on to animal experiments.

3) Statisticians have been consulted on experimental design to minimise the number of animals used whilst still obtaining meaningful results.

Refinement

Explain the choice of animals 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.

Refinement

The mouse provides a good model for various human diseases. The genetic and physiological similarities between human and mouse are significant thus the mouse provides a good model to study cancer biology.

We constantly review our surgical procedures to minimize the impact on animal welfare. We have recently changed how we carry out the surgical procedures in this study which will reduce the chances of post-surgical complications and also reduce the amount of time an animal is under general anaesthesia.

We use pain-killers to minimise any discomfort the animals might feel after surgery.

For all tumour experiments no animals will be allowed to suffer unnecessarily. No animal will be allowed to suffer from ulcerated tumours or any effects on movement, vision, eating, excreting or breathing. All animals will be monitored closely for signs of deteriorating health or suffering.

At present, for our surgical procedures, we make a cut in the skin in the shape of a letter ‘T’, but we are currently trying out the use of a single straight cut to see if that minimises scar tissue formation.

We use tissue from ear-clips which are taken for routine animal identification purposes to obtain the DNA we need to carry out genetic analysis. This means that we do not need to take any additional samples, such as from the tail, in order to get samples to carry out genetic analysis.

In addition, the animals will be housed in a facility, which is equipped with world-class equipment, and highly trained staff that regard animal welfare as a priority. The life of every mouse, including its health status, is captured in a bespoke database.

PROJECT 5

NON-TECHNICAL SUMMARY (NTS)

Project Title	Regulation of the actin cytoskeleton
Key Words	Actin cytoskeleton, filopodia, Xenopus
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The objective is to understand the molecular mechanisms of actin cytoskeletal regulation. It is unknown how cells attain their shape and move during processes such as wound healing, cancer cells spreading or embryos developing. The cytoskeleton inside cells is a network of filaments that give the cell its shape, which can be remodelled by the cell so that it can move. We want to understand how this cytoskeleton is changed to make different structures that determine different cell functions. To do this, we make extracts from frog eggs that contain all the molecules from inside a cell. We use these to grow the cytoskeletal structures outside the cell and observe them using a microscope. We can use these systems to identify previously unknown molecules and study how these, and the molecules we already know about, work together to regulate cell shape and movement. We may be able to benefit from others scientist's work that makes genetically altered frogs that are missing one of the molecules that we study, or have a fluorescent protein fused to it. We are also going to look at frog nerve cells that we grow in culture and observe how these same molecules behave in developing structures. We aim that these experiments will tell us more about neurodevelopmental disorders such as autism and Downs syndrome. In this work we may be able to benefit by using a genetically altered frog that has the cell membrane marked if made by other scientists.

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)?

Regulation of the actin cytoskeleton is a fundamental property of all cells. We are focusing on finger-like actin-rich protrusions called filopodia, which are particularly important in the brain for how the brain cells connect to each other and transmit information. Problems in how this happens are thought to underlie genetic conditions that cause intellectual disability such as Down syndrome and Fragile X syndrome. Problems in how the actin cytoskeleton is regulated are also thought to underlie autism and mental health disorders including schizophrenia and bipolar disorder. Regulation of the actin cytoskeleton is also important in cancer metastasis and immunity. Because we study fundamental biological processes, the variety of conditions means that there is a considerable disease burden that could benefit from our research. In the first instance our work will provide information which will help the worldwide community of cytoskeleton researchers. It may also help newly diagnosed people and families understand why genetic conditions linked to the actin cytoskeleton have the effects they do. In the medium term our work will provide new avenues for translational research in characterising where new therapies might be effective, in the last 5 years we already identified such an opportunity. In the long

term the whole body of actin research will feed into efforts to produce new therapies, at present our understanding is rudimentary.

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

Xenopus laevis, 860 animals over 5 years

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

There are no adverse effects expected from any of the procedures that will be undertaken. Making frogs lay eggs is a mild procedure; animals are returned to stock tanks and re-used afterwards with a minimum three month rest period.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

It is not possible to replace the egg extracts that we use with anything else for our cell-free assays. However these cell-free assays provide us with the important start point to allow us to explore our findings using other experimental systems instead of frogs. Important advances in recapitulating human biology and development in a dish mean that we can move into the use of human organoid cultures. We are also using advances in genetic technology and advanced microscopy to use *Drosophila*. Both these sets of work reduce the use of *Xenopus* for looking at filopodia in cells.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will ensure we use minimal numbers of animals by planning our experiments carefully, such as using more diluted extracts, sharing material between researchers within the group and continuously optimising how we perform the experiments and disseminating this information, such as in scientific methods papers. We are implementing measures to improve the quality of eggs laid and thus the usability of the material that we obtain. To do this we are keeping healthy young frogs and re-using them a maximum of 4 times. We would anticipate that any use of genetically

altered animals would decrease the overall numbers of frogs we would need to use as they make our experiments more efficient and easier to perform.

Refinement

Explain the choice of animals 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.

Refinement

The use of frog egg extracts is well established for studying cytoskeletal remodelling. Xenopus eggs are large and easily obtained which means that copious, concentrated, active egg extracts can be made from them with minimal animal suffering. The frogs are housed in grouped tanks with environment enrichment in a dedicated aquatic facility managed by animal technicians. The eggs are obtained by superovulation, which involves an injection of hormone to induce natural ovulation. Our animal technicians take special care of the animals during and after this procedure, helping them to feel safe by careful handling and monitoring them separately for 24 hours afterwards. The frogs are then returned to the colony after a veterinary surgeon has checked they can remain alive and rested for at least 3 months before being reused. Regular ovulation stops the frogs becoming egg-bound so is advantageous. We are starting to use non-GA frogs that are bred in-house to reduce stress on animals from transporting them from other facilities. In some cases, where we would be able to visualize endogenous proteins, use of genetically altered animals refines existing methods as it is more representative of normal biology. GA animals will be sourced from bona fide sources with permission to supply frogs to other licences. If in future we need to breed GA frogs then we would apply to add the necessary protocol.

PROJECT 6

NON-TECHNICAL SUMMARY (NTS)

Project Title	Mediators and effectors of pregnancy complications
Key Words	recurrent miscarriages, preeclampsia, preterm birth, gestational diabetes, inflammation
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Pregnancy should be a happy and healthy period for every woman. However, many women experience serious pregnancy complications that put at risk their lives and their babies lives. Miscarriages, preeclampsia and preterm delivery are some of these serious pregnancy-related disorders. Unfortunately, there is no available treatment or diagnostic method to determine which women will have complicated pregnancies.

To better understand the mechanism/s responsible for these adverse pregnancy outcomes we will use animal models that mimic the different clinical scenarios. Using these mouse models we previously demonstrated that miscarriages, placental damage and maternal vascular disease observed in preeclamspia and the cervical and uterine changes found in preterm delivery are associated with inflammation and the clotting system.

The aim of this project is to identify the specific molecules and cells responsible for the different pregnancy complications and thus identify targets for therapy and diagnostic methods. In addition, we will test treatments to prevent miscarriages, preeclampsia and delay the onset of preterm birth. We will also identify diagnostic, preventive and therapeutic strategies in congenital heart block and in adverse pregnancy outcomes associated with sickle cell disease. We will also investigate the long term health effects of abnormal pregnancies in the mother and offspring.

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 animal models will allow us the identification of the mediators responsible for adverse pregnancy outcomes and targets for prevention and therapy. If we validate these observations in humans, these molecules and targets could be used to detect pregnancy complications before they happen and develop new therapies to improve the health of mother and child. Using mice, we recently identified an effective treatment that was proven to be successful in women and several human babies were saved. We will build upon our recent success in preventing preeclampsia to continue our search for cures for other pregnancy complications. We expect to find treatments not only to protect the pregnancy and maternal health but also to allow a healthy development of the foetus and the prevention of long term health consequences for offspring.

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

We estimate to use no more than 10000 mice in 5 years.

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

The severity of the procedures described in this project will have minimal to moderate effects upon the animals involved. For example, light anaesthesia will be used for the non invasive imaging procedures. Small devices to deliver treatments will be placed under the skin to avoid the stress of daily injections using light anaesthesia. Pain killers will be administered. The vast majority of the animals in this project will not undergo surgery. All the procedures described in the different projects will be performed by skilled personnel and thus adverse effects are not expected. Animals will be closely monitored for any signs of distress. At the end of the experiments the animals will be humanely killed and after death organs will be taken for further studies to evaluate maternal and foetal health.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Unfortunately, we can not use computer models, or cell cultures to adequately replace animal models for pregnancy complications. Pregnancy is a phenomenon that only occurs in animals. Thus, animal models of pregnancy complications are essential. However, we will use cervical, myometrial and placental human tissue and cell lines to identify the molecules responsible for the changes that lead to adverse pregnancy outcomes.

Using a lower order species is not an option in our project as we need to study mammals with placentas in order to correlate results to humans. To study congenital heart block we will measure the contractility of the heart in zebrafish embryos.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To reduce the number of mice to be used we will use the following strategies:

1. Limit the group size to the minimum needed to obtain statistically significant data. I have extensive experience of using statistics in experimental design and we will make sure we use the minimum number possible to achieve statistical significance
2. We will perform multiple experiments simultaneously so that the same control group can be used for all experiments

3. We will try to collect as many different tissues from each animal so that additional animals are not needed
4. We will try to use new instrumentation/methodology that improves precision and reduces the number of animals. For example imaging techniques that are non invasive and allow the study of one mouse at different times instead of sacrificing them at predetermined times for studies along time

As principal investigator I always work closely with my students, postdoctoral fellows and junior scientists. I do not only supervise them but work with them. In my opinion, this close follow up is a very good way to prevent errors and thus minimize the number of mice used in experiments

Refinement

Explain the choice of animals 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.

Refinement

Because pregnancies have similar characteristics in humans and mice, mice constitute a good animal model. Mice are quick to reach sexual maturity (7- 8 weeks) and they have a short reproductive recovery (less than a week). Litter-bearing (6-10 pups/liter) is another advantage that allows the follow up of the offspring. Mice have short gestational period (≈ 20 days) and the reproductive tract is relatively small, thus it is possible to analyze the course of pregnancy in exquisite detail.

We looked and continue to look for procedures to reduce pain or distress in the animals that we will use.

1- Most of the studies designed to address the causes of pregnancy loss do not require surgical interventions as used by many other laboratories. Ee will induce preterm birth in mice by intravaginal administration of LPS, a non invasive model in which the maternal health is not compromised.

2- In addition, in the two mouse models of preeclampsia that we will study the relevant key features of preeclampsia appear spontaneously without surgical manipulation (e.g. ligation of uterine arteries) or the administration of any compound to induce the condition. Thus, by using these mouse models, in which preeclampsia develops spontaneously we avoid surgery and we minimise distress and pain

3- In the mouse models of pregnancy loss the embryos die at day 8 of pregnancy and in the preterm delivery model the fetuses born at day 16-17 are non-viable at this stage of development. Mice pups even at term have limited cortical development and hence limited or no perception of pain/distress.

4- Animals will be monitored frequently and closely for symptoms after administration of drugs or substances. skilled personnel will be responsible for the administration of the drugs.

5- We do not anticipate animal distress or suffering as the protocols we will use are very mild. However, if by any reason we detect signs of distress or suffering (e.g. lack of grooming, piloerection, hunched up or significant loss of weight compared to controls animals) mice will be humanely killed by schedule 1 protocol and protocols will be reviewed.

6- We will periodically check the scientific literature for new procedures to reduce pain or distress.

PROJECT 7

NON-TECHNICAL SUMMARY (NTS)

Project Title	Production, Breeding & Cryopreservation of GA Mice
Key Words	Breeding, Genetically altered, Cryopreservation
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

This project will create, breed and maintain rodents with genetic alterations (GAAs) and supply them for research into fundamental molecular and cellular functions and disease processes in the fields of biological, medical and veterinary science.

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)?

GAAs have made significant contributions to such research. However, the function of many genes, either individually or in the ways they interact to produce their intended effects, or how they are dysfunctional in disease, is still not known or is not fully understood. The use of animal models is necessary to determine these processes.

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

The expected use over a 5 year period is 23750 mice

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

The bulk of this Project will involve the breeding of GAA rodents, predominately mice. This will involve the natural pairing and rearing of rodents coupled with the need to take small samples to allow their genotypes to be established. The licence also allows for the generation, collection and subsequent implantation of embryos into recipient mice, this will involve a small surgical procedure. The majority of the mice >92% are expected to experience sub-threshold or mild severity and only a very small number may experience a moderate severity. The majority of these mice are expected to be humanely killed at the end of the protocol.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Many of the research projects will involve the use of *in vitro* systems such as cell culture, human tissue assays, computer modelling to complement the animal work. Such details will be expected in the justification for the animals' use to be reviewed by the AWERB.

In vitro assays cannot adequately model the complete array of molecular, cellular, physiological and behavioural interactions necessary to fully understand how genetic modifications result in normal or abnormal processes.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Unnecessary production or import of genetically altered animals will be avoided by searching cryobanks and databases.

Animals will only be bred if a user requirement has been established, and the breeding programme will be subject to regular review to optimally meet anticipated demand. Spare animals will be made available for use on other scientific projects.

Breeding will be optimised, wherever possible, to produce only the genotype required e.g. Homozygous breeding pairs.

Cryopreservation of gametes and embryos to archive lines will avoid wastage from the need to maintain colonies by continuous breeding.

Refinement

Explain the choice of animals 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.

Refinement

Mice are universally used for work involving genetic alterations. The standard protocols, methods and reagents have been optimized for this species and there are acknowledged benefits from their use.

The animals will be cared for by dedicated animal technologists who have the expertise and skills required in the breeding of the animals and are able to assess any welfare problems that may occur at an early stage and determine appropriate end points in consultation with the NACWO and NVS.

PROJECT 8

NON-TECHNICAL SUMMARY (NTS)

Project Title	Brainstem and spinal cord circuits
Key Words	neurogenesis, neuronal networks
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

To understand how the central nervous system controls homeostatic functions such as blood pressure.

To understand how neural stem cells in the adult spinal cord can be harnessed and controlled for therapeutic repair.

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)?

Manipulating the central nervous control of the autonomic nervous system is currently under investigation for several different therapies e.g. vagal nerve stimulation to treat heart failure. This work will investigate the circuitry and mechanisms underlying such approaches and may inform future trials. Even in the adult the spinal cord continues to make new cells from the division of stem cells. Manipulating the production and/or fate of these cells holds promise for future therapies. For example, in the spinal cord, conditions such as spinal cord injury, motor neurone diseases and multiple sclerosis may benefit from increasing cell production and promoting specific fates. On the other hand, these stem cells may also contribute to some CNS tumours and so in this case it could be beneficial to slow cell proliferation or promote fate to a non-dividing cell type. However, the mechanisms controlling stem cell division are poorly understood and it is vitally important to understand how their behaviour is regulated.

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

We expect to use 2550 rats and 8750 mice.

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

The majority of the procedures will carried out under non-recovery anaesthesia where the animal is killed by anaesthesia prior to organ removal. In some cases experiments will require recovery from anaesthesia following surgical procedures. Adverse effects are not anticipated and post-operative analgesia will be applied to limit suffering. At the end of experiments animals will be terminated for tissue collection.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The work will study how the CNS controls the autonomic nervous system and how spinal cord stem cells can be manipulated for therapeutic benefit. These will involve mammals which have similar circuits and cell types to humans which will help us translate our research to human benefit. Non-protected animal alternatives do not have such cellular organisation.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used will be minimised in several ways. Tissue from the same animal will be used for different objectives where possible. This reduces the total number of animals that would otherwise be required if a single animal was used for each project. We have also developed a method of storing tissue long term for future use which also reduces animal use as well as an organ culture for slices of nervous system that allows several tests to be conducted on tissue from one animal. In addition, each experiment is designed to maximise the amount of information gleaned since they often combine different approaches to verify this information.

Refinement

Explain the choice of animals 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.

Refinement

Rats and mice will be used since they contain cell types and assemblies of cells that are similar to those known in humans. Transgenic animals will be used when they make identifying specific cell types possible with fluorescent markers, helping to minimise numbers used.

Suffering of animals will be minimised as the majority of the procedures performed will have a minor severity where the animal is killed by anaesthesia prior to organ removal. In some cases experiments will require recovery from anaesthesia following surgical procedures. Post-operative analgesia will be applied in these cases to limit suffering of the animals.

PROJECT 9

NON-TECHNICAL SUMMARY (NTS)

Project Title	The function of the RASSF proteins and the Hippo pathway in tissue architecture and cancer.
Key Words	Tissue architecture, tumour formation
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The “Hippo pathway” is a signal that tells our cells to stop growing and proliferating. It is among the key signals that tell our bodies to grow to the right size and shape, but not larger or smaller. Disruption of the Hippo pathway in mice and people has been shown to lead to tumour formation. Although scientists are trying to create drugs that can restore normal Hippo signalling in tumours to stop them growing, these approaches have so far been unsuccessful. Our research is aimed at gaining a better understanding of this signal during animal development and tumour formation, and to identify new ways in which it can be targeted for patient benefit.

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

We hope our work will identify new diagnostic markers for early detection of cancers, as well as new therapeutic targets for drug discovery.

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

Based on the work we have carried out in the past 5 years under our previous licence, the number of mice we expect to use over the next 5 year period will be around 6000 mice bred and around 9000 mice used under the various experimental protocols.

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

The primary adverse effect of this work will be the development of tumours by some of the animals. The animals will be closely monitored by skilled staff in order to identify adverse effects as early as possible. The expected level of severity for all the experiments is either mild or moderate. The mice will generally undergo schedule 1 killing at the end of the procedures though for certain methods of tissue sampling or histological processing, some will be exsanguinated or killed by perfusion with fixative under terminal general anaesthesia.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We use other models such as fruit flies, mammalian cells in culture and computer models as much as possible to aid our research. However, the complex physiology of a tumour means that in order to gauge the likely relevance of our work on human health, we need to use mouse models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will reduce our animal usage by careful use of statistics to limit experiment size and the number of mice in our colony. Wherever possible, we will use the mice we breed to produce different types of data. We will constantly update our experimental strategy based on our work in flies and cultured cells.

Refinement

Explain the choice of animals 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.

Refinement

We have chosen to use mice for these studies for a number of reasons. They are ideal model organisms to investigate mammalian development and cancer - their biology is close enough to that of humans for our findings to be relevant to human disease. Mouse embryonic development and biology are well described, so we will be able to identify and characterise abnormalities easily. Also, the processes occurring during embryonic development and the emergence of cancer are very complex. There are many components (proteins, cells, and signals that pass between cells), which interact in ways that are poorly understood, and these interactions unfold over time. Currently, no *in vitro* system exists that is capable of accurately modelling these processes, therefore we need to study them in living animals. However, once we have some idea which kinds of processes are affected by loss of our proteins, we may be able to answer further questions using tissues or cells derived from the transgenic mice, rather than performing experiments on living animals.

All our work will be performed by skilled personnel who will prioritise animal welfare. Animals will be monitored on a regular basis to ensure minimal harm.

PROJECT 10

NON-TECHNICAL SUMMARY (NTS)

Project Title	Tumourigenesis and therapy
Key Words	Cancer, leukaemia, antibody, therapy
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Cancer remains a major worldwide health problem contributing more than 25% of premature deaths and consuming large amounts of health budgets annually. Furthermore, five-year survival for most common cancers has not markedly improved in the last few decades. This lack of progress in cancer therapy is partly due to nature of the disease where primary tumours move from the initial sites to remote places in the body making treatment difficult (metastasis). But also there is a recently recognised underlying feature that makes it likely that cancer will recur in a patient after treatment and this is the so-called cancer stem cell that resists drug treatment. We don't know enough about the biology of these cells. In addition, most common cancers show up when patients notice symptoms and they do not have a discernible pre-cancer stage that could be treated. An effective cancer treatment needs to take all these features into account

Our objectives are to understand how cancer starts and progresses and to implement a new generation of anti-cancer drugs. Our three objectives are linked by the concept that therapeutic targeting of the cells that initiate cancer will produce the most powerful therapies that will not allow relapse and cause the cancer to return. In particular our study of leukaemia formation and progression will tell us which cells start the cancers and which genes are important. If we know the genes that are important, and in turn their protein products we can implement our new intracellular therapy technologies to block their function. This will lead to new cancer treatments. A very important objective of our work in the lab has been to identify proteins on the surface of cancers that can act as anchors for nanoparticles that carry our new anti-cancer reagents. Thus for instance an immuno-nanoparticle in the blood stream with find its way to and lock onto cancer cells that have the surface protein. In turn, this will lead to the nanoparticle being taken into the cancer cell and releasing the anti-cancer therapeutic agent. We hope to use our work with animals to show the power and specificity of our combination of new technologies in cancer treatment.

We are anxious to translate our new anti-cancer therapeutic agents into clinical use and this is the major goal within the period of the new PPL.

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

We have developed a new type of anti-cancer drug that hits proteins inside cancer cells. We will develop ways to deliver these in the mouse pre-clinical setting, informing methods development to apply in treating of patients. The benefits that will arise is that we will have obtained data that can be applied to clinical development of new cancer therapy reagents. This will be in two main areas. First, identification and

characterisation new proteins on cancer cells for antibody therapy. Second, new drugs for cancer treatment.

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

We will be using mice. Over the whole project, we expect to use between 1000-5000 mice, excluding breeding colonies that will generate up 10000 offspring during the project. One purposes of our animal work are to identify the new cancer targets and develop antibodies to these proteins. We will need to immunise mice for this purpose and this work will extend over the period of the licence. We also will investigate the first cells that lead to cancer and in turn find new targets on these cells, in particular in leukaemias where the recurrence of disease after treatment (e.g. chemotherapy) comes from these initiating cells.

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?

Our work has three broad purposes. First, the study of cancer initiation and most mice will not have any adverse effect. A small proportion may develop leukaemia (<10%) and these will be humanely killed. We will develop ways to deliver macromolecules to mice and these will produce at most moderate severity. We do not expect adverse effects since the mice will be kept for short times (typically up to one week) after treatment before killing and tissue sampling. Any animals showing ill-effects will be humanely killed using Schedule 1 methods as the end of experiments. Where irradiation is used, a low proportion (<2%) will show acute infections or bleeding and all irradiated mice will be inspected from day 4 to-14 post-irradiation and in case of acute effects humanely killed using Schedule 1 methods. Mice that have been immunised will not usually display ill-effects. A small number (<1%) may show ulceration at injection sites and these will be humanely killed after consultation with the NVS.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Cancer development and treatment modalities can only be studied in animals and mice are the best species as transgenesis and gene editing are well developed methods in mice. However, all our methods are developed initially using *in vitro* tissue culture and 3-D models of tumour growth in culture where possible and are taken to the limits of usefulness in vitro. We do not use animals until we need to evaluate how our new anti-cancer drugs behalf in vivo. Further cancer initiation can

only be studied in vivo.
Part of our work is targeting proteins on cancer cells with antibodies. Whilst we plan to use immunisation of mice because we can obtain the best quality antibodies this way, we will use display systems such as yeast, mammalian cells and phage where practical in replacement technologies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Transgenic mice and gene targeting mouse strains are used, we keep them as homozygous strains to minimise pups produced and to obviate the need for genotyping that requires ear notching or tail biopsies.
Our main objective is the study of the earliest steps in cancer development so we will minimise the numbers of mice that are kept for periods exceeding 6 months
The nature of our anti-cancer drugs means that we can plan to carry out pilot analyses with small groups provided these are very precisely designed based on our tissue culture analyses and data in the scientific literature.

Refinement

Explain the choice of animals 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.

Refinement

We have to use mice as we have, for example, transgenic models that are humanized for genes expressed in childhood leukaemia that provide a target population. Because we are interested in tackling the pre-leukaemia stage (i.e. before the children show frank leukaemia), the mouse will not, in the main, have disease symptoms.
Genotyping, when needed, will be done with ear-notch biopsies.
Split dose irradiation for recipients to receive donor cells allows short recovery periods and conditional alleles that require inducers are advantageous as no phenotype exists prior to induction. The most refined route of administration is incorporating inducers into food or water.
Immunisation is the best way to obtain high affinity antibodies. We will restrict the use of adjuvants that have minimal side effects and Freund's complete adjuvant.
Where possible, we will use display systems such as yeast, mammalian cells and phage to develop antibodies.

PROJECT 11

NON-TECHNICAL SUMMARY (NTS)

Project Title	Arterial chemoreception; molecular mechanisms and functional role in health and disease.
Key Words	Chemoreceptor, Carotid body, Hypoxia, Carbon dioxide, pH
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

The carotid body is an arterial chemoreceptor, located in the neck, which senses oxygen, carbon dioxide and acidity in the blood. It is involved in the control of breathing and the cardiovascular system. The mechanisms by which the carotid body senses these variables are only partially understood. The basic outline is that hypoxia, acidosis or hypercapnia (high CO_2) generate an electrical signal in the membrane of a specialised receptor cell called the type-1 cell. This allows calcium into the type-1 cell which then promotes the release of neurotransmitters which excite nearby nerve fibres leading to the brain. Our understanding of how these stimuli generate the electrical signal in type-1 cells is incomplete. In particular there is controversy over how oxygen is detected in the first instance but there are strong indications that this is intimately linked to oxygen use by the mitochondria to generate energy. One of the main aims of this project is to determine how this oxygen sensor works and how it is coupled to the generation of an electrical signal.

Whilst the type-1 cells are intrinsically sensitive to hypoxia CO_2 and acidosis, their activity can also be modulated by neurotransmitters released from nearby nerves and circulating hormones. Defining how these pathways work is also critical to understanding what determines the sensitivity of this organ.

Chemoreceptor sensitivity can also be affected by environmental changes. For example its sensitivity to hypoxia (low oxygen) increases the longer it is exposed to it. This is part of a process called ventilatory acclimatisation which increases our level of breathing whenever we are exposed to hypoxia for periods of hours to days. Ventilatory acclimatisation to hypoxia is part of the process of acclimatisation to altitude and helps us to cope with the lower levels of oxygen in air under these conditions. Part of our research is aimed at trying to understand this acclimatisation response which is mediated through the oxygen dependent control of gene expression.

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 benefit that derives from this research is a better understanding of how this organ works, how its activity is regulated physiologically, how it can be controlled using drugs and how it adapts to environmental changes. This work is of interest to physiologists who seek a better understanding of the control of breathing and those who work on related problems in other tissues. It is also of interest to those who seek to understand disease states in which chemoreceptor dysfunction (over or under

activity) appear to play a significant role in affecting clinical outcome. Examples include sleep apnoea (periodic cessation of breathing during sleep), hypertension, heart failure and possibly type-2 diabetes.

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

Most of our experiments are conducted using tissue from rats but for some experiments we are likely to need transgenic mice. Over the 5 year period of this project we expect to use approximately: 1,400 wild type rats. 3,400 GM mice. 600 wild type mice.

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 our experiments are conducted in vitro / ex vivo using tissue removed from rats and mice. Owing to the anatomical location of the carotid body, and the organs high oxygen requirements we prefer to harvest these organs under terminal anaesthesia rather than use schedule 1 methods (in the interests of refinement). The expected severity of this protocol is classified as non-recovery, adverse effects would be no-worse than for animals killed by overdose of anaesthetic. Up to 3000 genetically modified mice will be bred with non-harmful phenotypes; severity level mild. Up to 400 genetically modified mice may be bred with harmful phenotypes with a severity level up to moderate. Up to 200 mice may be subject to unrestrained whole body plethysmography. This is a non-harmful non-invasive technique in which the animal is placed in a small chamber and its breathing is monitored by measuring small pressure changes in the chamber. These animals will be subject to brief changes in oxygen levels and carbon dioxide levels which are unlikely to have any long term adverse effects but may cause short term symptoms such as breathlessness, dizziness, headache & lethargy for the duration of the stimulus. Severity category is moderate. Up to 300 mice and 200 rats may be exposed to chronic hypoxia for 2-14 days. This may result in symptoms similar to acute mountain sickness in humans with loss of appetite, lethargy, headaches & fatigue. Severity category is moderate. All animals will be killed upon completion of the experimental procedures.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We are studying a highly specialised sensory receptor that measures oxygen, carbon dioxide and pH (acidity) of the blood. There are no cell lines derived from these sensory cells.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Reduction will be achieved mainly through careful tissue handling in *in vitro* experiments and careful planning of breeding programs for the production of transgenic animals.

Refinement

Explain the choice of animals 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.

Refinement

As we are studying a highly specialised receptor that is involved in the control of breathing we need to work with mammals. Fish may have receptors which have a similar function but it is by no means clear whether they are of the same embryological/evolutionary origin as those found in humans.

For *in vitro* studies we need to dissect out the carotid body and break it up into individual cells. We have found this process to work best in the neonatal rat. In this model the carotid body is a well-defined discrete organ which has relatively little connective tissue. This makes the organ easy to remove and disassociate into its individual cells using a mild enzyme treatment with the result that we get good yields of healthy cells that are highly responsive to stimulation. For some experiments we may need to use transgenic animals in which specific genes have been deleted. These experiments will be conducted using mice as appropriate transgenic animals, or embryonic stem cells, are already available.

PROJECT 12

NON-TECHNICAL SUMMARY (NTS)

Project Title	Clinical veterinary studies of naturally occurring disease in animals
Key Words	Companion animals, Clinical trials, Pain killers, Heart disease, Diabetes
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

As in humans, there are many diseases that occur in dogs and cats, which are kept as pets, about which we do not fully understand and for which we do not have effective treatments.

The aims of our studies are to investigate new ways of treating and managing diseases in companion animals, similar to clinical trials in humans with diseases.

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)?

Every animal included in our studies will have a disease which has occurred spontaneously. Their disease will be investigated thoroughly to work out the exact nature of their disease and they will be monitored closely to see whether they are getting better whilst on our studies: every animal will therefore benefit. The information gained from our studies will hopefully result in more effective treatments and better diagnostic tests being available for vets to use for the treatment of companion animals.

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

Up to 300 pet dogs and cats per year

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?

Every treatment or diagnostic test that will be studied will have undergone rigorous testing before our studies. This means that they will have been proven to be safe and we will be able to predict the type of adverse effects which might occur, so that we can detect and treat them early. Adverse effects are therefore predicted to be mild in severity. Non-specific adverse events can be seen with any clinical trial. These are usually mild and self-limiting including nausea, temporary loss of appetite, diarrhoea, constipation, lethargy and allergic reaction. Specific adverse effects related to the proposed clinical trials will also be predominantly mild and self-limiting and may include effects such as sedation, weakness and low blood sugar. If unexpected adverse events occur that are not mild, not self-limiting or require specific treatment, the animal will be withdrawn from the study, so that they can be cared for by a vet. We will not trial any treatment or test that, to the best of our knowledge, may worsen an animal’s disease and if any animal’s disease

deteriorates while on study, we will immediately withdraw them from the study, so that they can be cared for by their vet. Procedures to monitor the animals on clinical trials are expected to be associated with only transient, mild adverse effects including the transient, mild discomfort related to taking a blood sample. All animals enrolled to our studies will be pets. When the study is complete, we will make sure they have not been affected by the study and then return them to their homes.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The aim of the studies is to find new treatments for spontaneously occurring diseased in companion animals.
Once these treatments have been shown to be safe and are likely to be effective, the only way to know for sure if they will work is to conduct a trial in companion animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The numbers of animals used in our studies will always be the smallest number required to show a true result.

Refinement

Explain the choice of animals 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.

Refinement

All animals will be closely monitored at all times by vets and vet nurses: if any problems are seen, they will be
immediately withdrawn from the study to be cared for by a vet.

PROJECT 13

NON-TECHNICAL SUMMARY (NTS)

Project Title	Peripheral Pain Mechanisms
Key Words	Pain, Analgesia, Transgenic, Genes, Neurons
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Chronic pain conditions lead to substantial socioeconomic burden and ongoing suffering with pain can be detrimental for the quality of life of patients. Up to 28m people in the UK, or 43% of all adults, have been in pain for more than three months with the problem set to worsen as the population continues to age. Just one in seven adults under 25 reported chronic pain compared with almost two-thirds of people over the age of 75. Improved therapies for pain relief are still lacking and call for a better understanding of the physiology of pain processing in the nervous system to identify new drug targets.

Nerve cells in the peripheral nervous system are capable of transmitting painful signals, but we still don't know which specific cells are important for pain. This license covers a multitude of techniques applied to different animal models, which will allow us to explore the role of different types of sensory nerve cells with regards to processing painful inputs. This project will increase scientific understanding of gene products in pain pathways and in various types of painful conditions, as well as the determining factors underlying the transition of acute to chronic pain. Improved knowledge in the genetic components of pain processing are necessary for providing new drug targets that are more refined and specific for distinct pain syndromes.

Our aim, using the protocols and studies discussed in this project license, is to provide new analgesic targets and improve the way in which current analgesics are used to treat pain.

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 advance in our knowledge on nociceptive processing will shed light on how painful conditions can be treated. By understanding the genes underlying these changes in more detail, we will be able to design more specific and effective treatments for painful conditions in the future. The prevalence and impact of chronic pain is far reaching. A recent telephone survey of 46,394 people across 15 European countries and Israel illustrates the enormity of the current problem. Approximately one out of every three chronic pain patients reported that their pain was intolerable, and one in five reported being diagnosed with pain-associated depression or losing their job due to pain. Clearly, there is a need to investigate the causes and effects of chronic pain to find new forms of pain killers in order to better treat the substantial number of people affected by the condition. We believe that defining the particular sets of damage-sensing neurons in different pain syndromes will enable us to devise new focussed therapeutic strategies that have a better chance of success than the present very broad approaches to silencing neuronal excitability.

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

We expect to use roughly 20000 mice over 5 years.

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

Most of our protocols involve procedures of mild severity, except for a few exceptions which we have identified as falling into the moderate classification for the caution and protection of the animals. Our protocols are mostly based on models of human chronic pain conditions, so implementation of these in animal models is necessary for the advancement of our understanding and potential treatment of human suffering. Expected adverse effects might include postoperative stress or discomfort, but these will be quickly identified and measures taken to minimise suffering. In all these cases, or when unexpected clinical signs appear, we will consult our NACWO and NVS. At the end of each procedure animals will be euthanised according to certified Schedule 1 procedure and tissues will be isolated for further studies.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Pain is a whole body experience that necessitates studies in whole, intact animals. During this project we will investigate the role of potentially interesting genes involved in pain pathways and produce transgenic mice where this gene is deleted or silenced. *In vitro* cell culture can sometimes be used to replicate select aspects of neuronal signalling responses, but they fail short of providing an integrated, organism-level, physiologically intact environment in which such responses are normally coordinated. Initial work will, where possible, be investigated at a cellular level using culture systems. However, further investigation examining the connectivity of the gene products and understanding their greater role within the organism and during disease states can only realistically be replicated using live animal models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We carry out statistical power analyses for our studies, which allows us to limit use of animals to the minimum number in order to achieve our experimental aims. We ensure our study design (statistical analyses, any implicit bias) is addressed in order to maximise the scientific outcome of our work while ensuring the least number of animals are used. At each stage the same animals will be tested in multiple paradigms to minimise numbers used, and to maximise the data collected. For example, multiple behavioural, electrophysiology and imaging recordings can be achieved from one animal. A plethora of earlier research means it is now easy and routine to generate transgenic mice, which means fewer animals need to be used. Cell culture models and genetic data will also be examined for initial experiments (where appropriate), reducing the numbers of potential candidate genes and ensuring only the highest potentially beneficial research is taken into live animal models.

Refinement

Explain the choice of animals 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.

Refinement

Mice will be used for this project as they represent the most appropriate species for this type of work; they are very similar to humans genetically and physiological systems for pain are largely similar. Many key physiological systems and structures involved in pain processing were originally identified in rodent and other models, and validated in more recent human studies.

On top of this, decades of research has resulted in highly advanced and efficient techniques developed for investigating neuroscience questions in the mouse. Importantly, the mouse is highly amenable to genetic modification, allowing transgenic identification of specific cell types crucial to the fulfilment of the project.

Over several years of pain research, we have been able to establish protocols to minimise stress in rodents to ensure the best welfare of all animals and maximum scientific output from all studies requiring consistent animal handling. We have also developed new techniques to replace conscious animals with unconscious animals (i.e. in imaging experiments to assess cell activity) to reduce suffering.

PROJECT 14

NON-TECHNICAL SUMMARY (NTS)

Project Title	Production of Genetically modified Animals (Service Provision)
Key Words	Genetic Modification, Service, Mouse
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Creation and importation of genetically modified mice for cancer research, diagnosis and treatment

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)?

To understand cancer cell gene mutation, initiation and development. Potentially to develop human cancer diagnosis and treatment.

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

Mouse Total number in 5 years: 16,400

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

The severity of the majority of animals used in this licence is mild, such as hormone dosing and transgenic breeding. Others are used as embryo recipients and Vasectomised males after surgery, which will experience a moderate severity. In some circumstance, a transgenic line has a moderate severity will be bred under this licence and those animals will be transferred to the user's licence as soon as possible after creation OR rederivation.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Transgenic animals are the most reliable disease model for studying and developing treatment for cancer. All requests to create new lines had 3Rs assessed during their PPL applications.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Transgenic Service is keen to develop new transgenic technology to reduce the usage of animals. Now we are using CRISPR-CAS9 technology to produce GM animals more quickly and fewer animals are used.

Refinement

Explain the choice of animals 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.

Refinement

Mice are widely used for cancer modelling and there are many lines already in existence. This licence enables us to create and import lines to study different types of cancer and genes.

PROJECT 15

NON-TECHNICAL SUMMARY (NTS)

Project Title	Discovery of therapeutic antibodies
Key Words	Cancer, Antibodies, Toxin, Immunity
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The objective of this project is to enable the discovery and development of novel antibody fragment-based therapeutic agents for cancer. The treatment of cancer is undergoing great change and antibodies are playing a key role in many new and exciting therapies. For example, antibodies capable of enhancing the immune response to tumours (Immuno-Oncology (IO) antibodies) are leaving some patients with advanced cancers in long-term remission (some considered ‘cures’). However, this is true only for a small subset of patients and cancers. Similarly, antibodies can be used to deliver highly potent toxins to tumours. Such Antibody Drug Conjugates (ADCs) are very good at killing the cancer cells but their physical properties mean that it is often difficult to give enough drug to patients before they experience treatment-limiting side effects of the toxin. This project will be used to create the next generation of antibody-based drugs capable of addressing many of the issues associated with current IO antibody and ADC drugs. It is hoped that this will bring the opportunity of long term remission from disease to a larger number of patients than can currently benefit by creating new drugs capable either of enhancing the immune response to cancers or delivering toxin more efficiently to cancer cells. The data generated by this project will allow us to identify and develop new ways of treating patients with incurable diseases.

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 potential benefits of this project will be the progression of new therapies into clinical development and ultimately onto the market bringing benefit to patients. We intend to bring one, perhaps two new therapies for cancer into clinical development over the next 5 years.

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

6500 mice over 5 years.

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

The mice are not expected to have any severe adverse effects from immunisation. Immunisation can occasionally lead to mild symptoms such as local non-painful swelling. If any adverse effect exceeds the mild category or is not temporary, the animal will be humanely killed. At the end of an immunisation schedule, all mice are humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Immunisation allows mice to develop a diverse antibody response to a specific target. This antibody response matures over the course of immunisations to mount a response containing high binding human antibodies. This process cannot be achieved in vitro.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Study cohorts will be kept to a minimum size. This has been determined in previous studies as the minimum number required to provide a diverse repertoire of functional antibodies. Study design will consider any prior knowledge of the target to enable successful outcomes and therefore fewer studies. Breeding the mice will be co-ordinated with studies to limit the numbers being bred.

Refinement

Explain the choice of animals 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.

Refinement

Humanised mice have proven to be suitable for drug discovery programme. Immunisations have been tested and responses monitored to identify the protocol involving few injections and achieving high antibody titre. Adverse effects are not expected but animals will be monitored closely after all procedures.

PROJECT 16

NON-TECHNICAL SUMMARY (NTS)

Project Title	Study effects of anti-cancer agents on tumours
Key Words	CAM, angiogenesis, Chick-Embryo, anticoagulants, DOACS
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The main aim of this work is to refine our understanding of how anticoagulant drugs interfere with the complex processes of the development of new blood vessels through their effects on the blood clotting cascade and how this disruption of vessel formation impacts tumour growth and development.

The development of new blood vessels (known as angiogenesis) is a key driver of growth and spread of cancer. By knowing more information about how to interfere with angiogenesis, would help us to define better strategies (which could include drug combinations) to block this key cancer process. By doing so we would like to move from a generic anti-angiogenic approach to a more ‘individualised' approach to these treatment strategies.

This will be carried out in 2 steps. The first step is testing the effect of anti-coagulant drugs on vessel development alone. Then, secondly, developing a model of tumours grown in the laboratory and implanted into a particular membrane system of the chick embryo (called CAM) that can be visualised and photographed. The aim is to test the ability of the anti-coagulant drugs to slow tumour growth by inhibiting the development of vessels that supply the tumour.

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)?

From our previous work to date we have established a successful model which was effective and reproducible in showing the anti-angiogenic effects of a conventional anti-angiogenic agent and also of an anti-coagulant usually used to treat blood clotting. This model will be used and refined further to study the contribution to angiogenesis of the specific proteins or groups of proteins inhibited by the new anti-coagulants. These are drugs that each inhibit just one specific protein in the blood clotting cascade. We are in a fortunate position of having established collaborations with two companies that produce anti-Xa inhibitors and are negotiating with the third for access to their anti-thrombin inhibitor. We will be comparing these findings to the effects of the specific anti VECF or PDGF inhibitors that are currently in used in clinic as part of cancer chemotherapy treatments. If it is found that these new direct anticoagulants have a comparable action then there is potential for them to be used within the drug combinations used to treat cancer. There is also scope for further expansion in this field as there are at least 3 new agents in development phase that target other distinct pathways of the coagulation process and should they successfully get to market this should give us a means of studying how the other areas of the coagulation pathway interact with the vessel development process. The second part of these experiments will use model tumours grown in the laboratory

and implanted into the chick embryo visualised membrane system with the aim of using them to study interaction of developing tumours and coagulation system. There is a chance that different proteins are involved in vessel formation in different types of cancer. If this is the case we may be able to identify which agent would be most effective against specific tumour types leading to a more ‘individualised' approach to these treatment strategies. Finally the development of using model tumours grown in the laboratory and implanted into the chick embryo visualised membrane system will benefit animal experimentation overall by reducing the need for rodent models. The chick embryos are not sentient and feel no pain and thus remain an excellent alternative to rodent models. Moreover, the anti-coagulant agents and tumours are placed onto the chick embryo visualised membrane and not onto or into the body of the chick making the procedure much less invasive and harmful than other methods of studying tumour vessel interactions.

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

Based on data gathered from Kayaga et al., 1999 (11) we have found that individual groups of between 5-8 animals was adequate to allow statistical comparisons between control and experimental agent. As we are proposing to carry out a full range of experiments on the three different anti-coagulate agents we expect to use ~350 eggs/years for these anti-angiogenesis experiments. We are also planning to be involved in collaborative work on a new type of implantation experiment using model tumours grown in the laboratory and implanted into the chick embryo visualised membrane system. We expects to use ~150 eggs/years for this work therefore totalling ~500 embryos/yr for all experiments.

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?

There are 2 possible sources of adverse effects; toxicity due to drug treatment and micrometastases from transplanted tumour cells. Micrometastases are when the cancer spreads from its original location on the membrane to other sites potentially within the body of the chick. We observe the eggs every 24 hours for signs of life. After embryo development day (EDD) 14, the chick is deemed to be sentient and the chick moves around, stretches its legs etc. Any changing in normal movement indicative of toxicity due to drug treatment will lead to immediate termination by schedule 1. Micrometastases are unlikely to cause pain and suffering as grafting a tumour doesn’t impact the day to day behaviour of a developing chick. However in the very unlikely situation of the observation of overnight rapid tumour growth, this may result in the chick incurring a tumour size that would cause distress before the next 24 hour observation and as such is grounds for immediate termination. The maximum volume of tumour allowable will be 100 mm3.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

At present there is no replacement model to allow the study of angiogenesis other than an in vivo setting. We have, and use, some artificial models that study the flow of blood factors/components and can simulate some of the effects of blood coursing through a vessel. We also have, and use, models that allow us to study the movement of endothelial cells in a matrix. But there are no artificial model replacement of studying the complex 'sprouting' of new vessels (which includes interactions of a number of cell categories (endothelial, muscle fibroblast etc). and the interaction between tumours and vessels. The model we have developed is the lowest one possible to give useful and robust results on differential agent effects and compared to others (such as mouse fenestration experiments) causes no perceptible distress to the animal (Chicken Embryo)

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

In terms of use of embryos (eggs), the main losses are incurred during delivery and also in the first week of incubation (prior to the opening of the egg). In addition, we will invest in a small commercially-available incubator capable of holding and rotating up to 40 eggs. This will prevent any losses as a consequence of stasis of the eggs. We envisage that these measures will minimise any unnecessary losses and reduce the number of embryos needed to ensure that we can perform the studies appropriately.

From the work to date we have concluded that although we were able to 'grow tumours' of patients with melanoma and ovarian cancer and verify that the tissue was pathologically indistinguishable from the primary cancer, our initial goal of expanding the technique to study 'tissue poor' cancers such as lung and pancreatic cancer was limited by two major factors. The first being that the smaller the tissue implanted in the CAM the more variable the successful 'take' (engraftment) was in the embryo but even when successful engraftment was seen, the limited growth period in CAM (2 weeks) did not allow sufficient enlargement of the cancer to allow further implantations. So studies of the growth and anti-implantation effects of the anti-coagulant agents we are working with could not progress and therefore no further animals will be used in this direction.

Refinement

Explain the choice of animals 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.

Refinement

Preliminary experiments to optimise techniques as described in the licence have been completed. We understand differential growths of the cell lines we plan to use and the inocula sizes needed for successful tumour generation. There will be no further experiments with solid implanted tumours directly from patients.

Assessment of sterility of technique has shown little attrition due to infection. For the antiangiogenesis experiments of agents (e.g LMWH), the optimisation of volume of matrigel, implant day, treatment day and culling have all been completed and the small gelfoam sponge technique works well with minimal animal attrition. This work has enabled us to refine our experiments so that the vast majority of the experiment will be finished by EDD 14 (rather than EDD 20 in our previous protocol) so reducing the embryos exposure to agents.

We will be collaborating with experiments to using model tumours grown in the laboratory and implanted into the chick embryo visualised membrane system. It is likely that some of the optimization experiments described in the previous licence for human tumour implants will also need to be undertaken when this technique is transferred to a CAM setting.

PROJECT 17

NON-TECHNICAL SUMMARY (NTS)

Project Title	New treatments for glioma
Key Words	brain, cancer, glioma, radiotherapy
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Gliomas constitute a group of brain cancers, of which glioblastoma (GBM) is the most common and deadly type in adults. Despite the best treatment currently available (surgery, radiotherapy and chemotherapy), most patients survive just 12-15 months after diagnosis. This project aims to address the desperate need for new treatments that can improve outcomes for this devastating disease.

The specific objectives of this project are to identify promising new treatments, particularly those that can be used to improve the effectiveness of radiotherapy in patients with gliomas. The focus of this work is on assessing new drugs that increase the toxic effects of radiotherapy on the tumour and thereby slow or prevent tumour growth following treatment, either by preventing cells repairing damage caused by radiotherapy or enhancing the activation of local immunity.

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 also contribute to a better understanding of how or why some drugs work with radiotherapy, both in the tumour and in surrounding normal tissue. This will help define which types of drugs may be useful in treating gliomas. This work will provide robust pre-clinical data that will underpin design of early phase clinical studies for patients with poor prognosis brain tumours. By matching the sorts of treatments that can be carried out in the clinic we will provide evidence for which drugs or combinations should be tested in patients as well as justification for rejecting ineffective or toxic treatments. We will work with academic partners as well as pharmaceutical companies to take these findings into early phase clinical studies. We do not expect all of the interventions that we test to be effective, but all of the experiments will contribute to our understanding of which approaches are effective and why. In all cases we will maximise the opportunities to interrogate the relevant biology using imaging, tissue and blood based biomarkers that will support our main outcome data and also permit more efficient design of future experiments and clinical studies.

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

We will use only mice and expect to use approximately 1500 animals over 5 years.

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

Animals are housed in individually ventilated cages with free access to water and food according to current guidelines. Human GBM tumour cells will be implanted

under the skin or directly into the brains of mice. The latter is done under general anaesthetic by inserting a needle through a small hole drilled in the skull. Surgery takes no more than 30 minutes per mouse and animals recover from anaesthesia within one hour. Risk of infection is minimised by maintaining sterile conditions during surgery and administration of antibiotics. Pain-killers are given to reduce the temporary discomfort caused and mice should be fully recovered and behaving normally within 24 hours. Tumours growing under the skin can be measured directly and various non-invasive imaging methods, such as CT or MRI, will be used to monitor tumour growth in the brain. The latter are performed under general anaesthetic. Radiotherapy will be delivered using equipment that closely mimics the procedure in cancer patients, including a CT scan to identify the target region. Side effects are negligible due to the ability to target the radiotherapy to very precise areas (i.e. tumour only). Radiotherapy is also performed under general anaesthetic. Anti-tumour treatments will be delivered by a variety of methods: by mouth (via a feeding tube) or by injection under the skin, into a vein, into the abdominal cavity or directly into the tumour. All of these methods cause no more than temporary discomfort and no lasting harm. By defining appropriate doses in non-animal experiments, the treatments themselves should produce only mild and temporary side effects at worst. In some experiments we will test a range of doses of agents that have not been assessed in these animals before, therefore some may cause more significant toxicities. These experiments make up a very small proportion of the planned work and will involve small numbers of animals in each case. Even in these experiments we would expect most animals to experience only mild or moderate toxicity. Expected toxicities include; Ulceration of tumours growing under the skin, which can also impede movement. Tumours implanted in the brain may result in behavioural symptoms such as hunched posture, reduced mobility, isolation and weight loss. Animals will be examined at least once per day for signs of ill health or the side effects of treatments. A scoring system will be used to assess all aspects of well-being and any animal reaches a predetermined threshold, it will be humanely killed to prevent unnecessary suffering. At the end of experiments, animals will be humanely killed and the brains/tumours will be recovered for further analysis.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We will do as much preliminary work as possible using in vitro cultured GBM cells, to select the most effective agents and establish appropriate doses.

However, animal models are currently the best way to recreate the complex environment of tumour development, which can have a significant impact on the effectiveness of any treatment.

In additional, data from animal models is required by regulatory and research bodies for translation of novel therapies to early phase clinical trials.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Statistical analysis, including power calculations, will be used to determine the minimum numbers of mice used while ensuring sufficient data is generated to produce meaningful results.

Non-invasive imaging techniques in live animals and analysis of tissue samples collected post-mortem will allow us to maximise data collection during and after experiments and reduce the total number of animals required.

Refinement

Explain the choice of animals 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.

Refinement

Animal models using patient-derived cells represent the most accurate way of recapitulating the development of GBM in humans and generate more meaningful data. Mice have been shown to recapitulate the human disease accurately in pre-clinical experiments.

Use of state of the art equipment, such as SARRP for radiotherapy, will allow us to perform more clinically relevant experiments and minimise side-effects.

Non-invasive imaging will allow us to monitor tumour growth more closely and anticipate the onset of symptoms associated with tumour burden.

Frequent careful monitoring and recording of the health status of all animals will serve to prevent unnecessary suffering. As far as possible we will design experiments, procedures and end-points associated with the mildest possible endpoints.

PROJECT 18

NON-TECHNICAL SUMMARY (NTS)

Project Title	Engineering synthetic vectors for various gene therapy applications
Key Words	Gene therapy, Synthetic vectors
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The scientific basis and justification for this project is in pioneering the translation of nanotechnology to preclinical gene therapy. Our focus is to perform basic chemical biology and nanotechnology research, and then transform knowledge gained into successful new therapies with a specific interest in genetic therapies.

The overall goal of this project is to generate new gene delivery systems for efficient gene transfer *in vivo*, with therapeutic efficacy in varied disease models.

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)?

Despite initial promise and substantial progress in gene therapy research, human gene therapy is still based on technologies that so far do not allow for routine clinical use. Safety and efficacy in gene therapy depend upon selectively delivering the therapeutic gene to the correct tissue without inducing harmful side effects. Due to complications associated with immunity, toxicity, limitations in dose, and difficulties in scaling operations for mass clinical production, viral vectors do not offer the best qualities for use in clinical gene therapy. By focusing on the development of synthetic and hybrid vectors with obvious potential for large-scale production, we hope to see the technology developed in our lab applied on a mass scale in a clinical setting for the treatment of any number of genetic and acquired diseases in man. Therefore we expect that the benefits of this research to be substantial as we have demonstrated the benefits of using viral and in particular non-viral vectors for the treatment of genetic diseases including atherosclerosis, cancer, stroke and Parkinson’s disease, and hopefully for musculoskeletal diseases too. The prevalence of all of these diseases is rapidly increasing worldwide and therefore seeking new effective therapeutics is of extreme importance. In particular, degenerative diseases such as Parkinson’s disease and musculoskeletal conditions are beginning to have a huge socioeconomic impact in the Western world due to the largely aging populations. Therefore the development of effective therapeutics would not only benefit patients on an individual day-to-day basis but also at a higher level both socially and economically. Despite the high cost of research and development of these types of therapeutics, it is of upmost interest to the public and society as a whole, and would in the long term have an impact as an economical necessity

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

Both mice and rats will be used to achieve our 5 year project objectives. In total to achieve our 9 project objectives, 19,400 animals will be used (more specifically 14,400 mice and 5000 rats).

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?

Preliminary experiments to determine the biodistribution, biological activity, therapeutic potential and safety will be performed in healthy animals. Further experiments will be performed in diseased animals to explore the therapeutic potential of these new gene therapies. Different disease models will be used to explore potential new gene therapies, including cancer, atherosclerosis, Parkinson's disease and skeletal muscle injury. All of these disease models are of moderate severity. Animals will experience some discomfort, distress or pain, however all steps will be taken to minimise these adverse effects, including compulsory pain relief. The earliest end-points will be chosen to cull the animals in order to minimise any actual/potential suffering to the animal. Humane methods of killing will be used.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

It is an unfortunate truth of non-viral vector mediated gene therapy that *in vitro* data does not shed much light on the *in vivo* performance of a specific vector. However, much of the previous work in synthetic vector development has focused on transfecting cells *in vitro*. There have been very few thorough studies characterizing new vector functions *in vivo*, leaving little chance for any clinical study using most of these vectors.

While we will make every effort to study *in vitro* the efficiency of gene expression, which depends upon the escape of the nucleic acid within the synthetic vector from the endosome into the cytosol after the vector has entered the target cell, it will still be necessary to study the uptake and distribution of these vectors, as well as their immunogenicity, in animals. Biodistribution and gene expression are complex outcomes that depend on interaction of the vector with blood, the immune system, and multiple tissues. No *in vitro* systems come close to this kind of complexity.

Biodistribution, gene transfer efficiency, and long term gene expression studies could theoretically be conducted in numerous small animal models. Mice are chosen for this study for a variety of reasons, primarily as they are the least sentient species that give data applicable to human clinical studies. Additionally, the mouse is used for numerous gene therapy studies as its response to a variety of traditional gene transfer vectors and reporter genes is well characterized. Immunocompromised mice are sometimes required when human tumour cells are used. More specifically for tumour studies, tumour growth (flank or metastatic) is dependent on several

features of the living animal, e.g. tumour/host stroma interactions, angiogenesis, organ micro-environment, etc., which cannot be fully replicated *in vitro*. Therefore, in order to translate findings towards the clinic, *in vivo* modelling is essential.

Numbers of animals to be used will be minimized by thorough evaluation of each proposed vector *in vitro* where feasible and/or appropriate, and by engaging in stepwise experiments – if a vector fails to show promise in early studies, it will be dropped from further *in vivo* characterization until it has been reformulated to improve performance. By running experiments in parallel with well characterized vectors, control groups can be used for multiple experiments.

In terms of *in vivo* Parkinson's disease modelling, both mice and rats can be used, although rats are the preferred animal model since the 6-OHDA induced hemiparkinsonian rat model is the most well-established. Some *in vitro* Parkinsonian models have been established although they do not truly reflect the complexity of the disease and have many limitations. Therefore, rodent models of Parkinson's disease are essential in assessing *in vivo* efficacy of gene therapy vectors. Furthermore, behavioural testing can be performed in rodents to assess therapeutic efficacy of gene therapy vectors; a crucial read-out that cannot be obtained from *in vitro* modelling. Each experimental group will consist of 6 animals which have been consistently found to be an effective minimum for statistical analysis of data resolution of significant results. A crucial factor which will be considered is the data/animal ratio which is greatly dependent on the variables assayed for *ex vivo.* These will be as extensive as possible and samples may be fixed or frozen to enable subsequent assays in the future.

Similarly, for the rodent skeletal muscle injury model there are no suitable alternative *in vitro* models available and functional/behavioural testing will be performed to assess therapeutic efficacy of gene therapy vectors at regenerating injured skeletal muscle. Therefore *in vivo* modelling is essential for this purpose, although animal numbers will be minimized where possible.

For work regarding implantation of devices, pre-assessment of probes can be carried out in both cell culture and *ex vivo* brain slices. This early work can help to assess short term impacts of the probes. This can give initial information, however, a full *in vivo* model is necessary. This is particularly relevant when considering complex neurological disorders such as Parkinson's disease and epilepsy which involve multiple brain regions and their interactions, which are far too complex for an *ex vivo* model

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

All steps will be taken to minimise animal use and to obtain as much information from fewer animals, thereby reducing the future use of animals. One major area where we will reduce the number of small animals used is by using live non-invasive, whole body imaging. Techniques that will be applied are X-ray, CT, SPECT, PET, MRI, MSOT and IVIS imaging which will all help to reduce the number of animals used in basic/translational research. The same animal will be imaged multiple times in order to monitor visually, usually in real time, the progression/regression of disease (e.g. tumour growth rate) or to track the biodistribution and residence time of gene therapy vectors both in healthy and diseased animals. Utilising imaging techniques will help avoid the need to sequentially sacrifice animals at different time points, ultimately allowing for significant reductions in the number of animals used per study.

Furthermore, improved experimental design and statistical analysis will allow for further animal number reduction. More specifically, by choosing a suitable experiment design (e.g.: completely randomised, randomised block, Latin square, etc.) and deciding on the statistical analysis before starting the experiment can allow for further reduction of animal numbers, bearing in mind that statistical methods may be modified when the results are obtained.

The design of the device implantation work is to have recording or stimulating devices in place during disease set up and progression, increasing the information obtained from each animal. As well as this, imaging techniques can be carried out longitudinally to gather more information from a single animal and prevent culling more animals at different timepoints.

Refinement

Explain the choice of animals 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.

Refinement

For cancer related studies, flank tumours will be considered our first-line model, when possible, in order to minimise the need for the metastatic type and its associated adverse effects. Both targeted and non-targeted vectors will be used here. Metastatic tumour models in immunocompetent mice will be used for testing targeted vectors with long circulation time (shown from flank models) in order to test their ability to reach the tumour site and achieve the required therapeutic effect. Metastatic type will be used as a first choice only when tissue characteristic (i.e. lung or liver) is a prerequisite for the therapeutic mechanism (i.e. bearing specific

receptors in targeted delivery). Animals will be culled before the development of severe side effects.

Toxin-induced models of Parkinson's disease (6-OHDA and LPS) will be used to assess the therapeutic efficacy of novel gene therapy vectors for modifying the disease process and the ability to offer neuroprotection. The 6-OHDA induced hemiparkinsonism model is the most well characterised and reproducible model of Parkinson's disease in rodents, both at the pathological and functional (behavioural) level. Similarly, LPS-induced parkinsonism is also well characterised, particularly in mice. The reproducibility and consistency of these moderate severity models of Parkinson's disease will enable us to reduce the number of animals needed per study but also improve the quality of data obtained both at the biological and behavioural levels.

For the epilepsy model, refinement will include, wherever possible, housing epileptic animals in pairs. However, due to the nature of the model, which is often associated with increased aggression, single housing may be deemed the most appropriate situation to minimise stress. If single house is the case, environmental enrichment will be a particular consideration to ensure all animals have the highest levels of wellbeing to suit their specific needs.

Both the models of atherosclerosis and skeletal muscle injury will be achieved by performing either chemical or mechanical injury to the blood vessel or muscle, respectively. Pilot studies will be performed for both disease models to determine by which means (either chemical or mechanical insult) achieves the most reproducible pathology and symptoms of disease but also with the least suffering and distress caused to the animal (including mortality rate).

The refinement of all scientific procedures and maintaining a high-level of husbandry will help minimise the actual/potential pain, suffering, distress, or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. Refinement of all procedures will not only benefit the animals but will also improve the quality of our findings, increasing reproducibility and reliability of all results. First of all, non-invasive techniques will be used where possible/suitable, for example: choosing the least painful and stressful route of administration of gene therapy vectors, considering the volume and frequency of administration too. Wherever necessary appropriate anaesthesia will be employed to further reduce the suffering and distress caused to experimental animals. Appropriate analgesic will always be used for pain relief and for all protocols the earliest endpoints will be used where possible. After each procedure is performed, animals will be closely monitored for adverse effects (mild, moderate, severe) and appropriate steps will be taken to minimise suffering, pain or distress. For example, shortly after inducing Parkinson's disease, sometimes the rats are less mobile and are unable to reach food in the hopper of the cage, therefore food pellets and hydrogels are placed inside the cage to prevent disruption to feeding, weight loss or dehydration.

In most cases approved Schedule I procedures will be used to cull animals, however, on occasions perfusion and fixation procedures may need to be performed in order to preserve the tissues for post-mortem analysis. On such occasions, animals will be given a suitable dose of non-recovery anaesthesia in order to minimise the suffering/ pain felt.

Before any procedure is performed, all animals will be handled in order to familiarise them with the researcher, thus allowing for voluntary co-operation with procedures like blood sampling and behavioural testing. This will allow the researcher to have greater control over the procedure and both the handler and animal will be less stressed, plus better quality data will be achieved.

Furthermore, by maintaining clean and spacious accommodation for the animals, and enriching their environment so that it meets the animals' physical and behavioural needs, for example: providing nesting opportunities for rodents or the opportunity to climb and hide, suffering and distress caused to the animals will be reduced.

PROJECT 19

NON-TECHNICAL SUMMARY (NTS)

Project Title	The role of ER stress in inflammation
Key Words	Arthritis, Stress, Inflammation
Expected duration of the project	0 year(s) 4 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:
No	(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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

IL-23 is an important protein in the immune system, although it has a useful function in fighting infections, it can also affect immune system cells so that they cause tissue to become inflamed and so induce arthritis. Identification of factors that regulate the appearance of IL-23 in the body are of significant clinical importance.

[REDACTED] We wish to extend our licence by a short period to examine whether this stress protein is affecting the expression of IL-23 in areas of the body that are known to be important in contributing to the development of arthritis and inflammation.

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

This research is particularly relevant to developing new treatments for ankylosing spondylitis, and related forms of arthritis diseases. Ankylosing spondylitis is a severe long-term (chronic) condition which affects people in their twenties in which the spine and other areas of the body become inflamed. The SKG animal model that we will use in our research has the advantage that it mirrors the human disease extremely well. This means the SKG animal model can be used to illuminate how the human disease is occurring and how it can be treated. This research is particularly relevant to developing new treatments for ankylosing spondylitis, and related forms of arthritis, where IL-23 is known to play an important role.

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

We will use mice strains that are susceptible to induction of experimentally induced arthritis. We will compare the induction of arthritis in mice of the same strain that have also been genetically modified. We will need to breed around 400 mice to produce 100 mice of the correct genetic background for our study. The work will be over a period of 4 months.

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?

Mice bred for this project live normally and do not exhibit any adverse effects resulting from the loss of a stress protein. Mice that that then undergo an arthritis inducing protocol will have inflamed joints but are able to move normally and forage well. Any mice that exhibit excessive arthritis or distress are humanely culled. This ensures that the severity does not reach a level where the mice behave abnormally, or suffer excessive pain. At the end of the experimental procedure all the mice will be

humanely culled and the tissue from different sites in the body will be examined for inflammatory markers

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

This project is designed to provide essential validation of results obtained using cultured human cells. We have only used mouse models that are supported by our initial work using cells in culture. This has ensured that mice do not unnecessarily go through arthritis inducing protocols. Our cell culture work has provided strong evidence to support the use of the mouse models we have proposed. Because the immune system is a complex pathway affecting diverse cellular processes, the reliability of simple cell culture models for outcome of a given genetic or pharmacological manipulation is limited and the use of an animal model is the next important step to understand the role of a stress protein in the body.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The animal strain that we propose to use is predictable and less variable than other models of arthritis and allows us to use smaller numbers of animals to obtain statistically significant results.

In our previous licence we have produced experimental arthritis in mice and these have served as the basis for calculations to define the correct number of mice to be used that provide data that is scientifically valid.

Refinement

Explain the choice of animals 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.

Refinement

The majority of our work will involve examination of inflammatory markers on tissue and cells isolated from mice that have been culled at early time points and so prevent the mice from experiencing the moderate signs of arthritis.

PROJECT 20

NON-TECHNICAL SUMMARY (NTS)

Project Title	Brain Cell Networks in Epilepsy
Key Words	Epilepsy, Networks, Treatment, Optogenetics
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Anti-epileptic drugs and surgery to remove epileptic tissue are often ineffective in controlling seizures in patients with epilepsy. Furthermore, these treatments can have negative side effects as these interventions are not always specific to brain cells that start seizures, medications often affect the entire brain and surgical removal of epileptic tissue may damage neighbouring areas. Therefore, new therapies are needed that will specifically block seizures while minimally affecting the rest of the brain.

We will evaluate strategies to block seizures through manipulation of brain cells that send connections specifically to seizure generating brain regions in epilepsy. We will test the use of a new technology, called optogenetics, which allows us to control brain cell activity with light and attempt to block seizures in animal models of epilepsy.

Our general plan is to 1) map brain cell networks involved in the generation of seizures in animal models of epilepsy, 2) determine whether manipulating the activity of specific cell types in these networks through the use of optogenetics can reduce seizure activity in epilepsy models and 3) identify which type of light stimuli are the most effective at blocking seizures (e.g. continuous stimulation or light at specific times).

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 findings from this study will potentially lead to a new approach to reduce seizure incidence which may be applicable to patients within 5 to 10 years. The study will also increase our knowledge of brain circuitry in epilepsy, which can improve other treatment strategies that target specific brain areas, for example gene therapy or deep brain stimulation.

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

We will exclusively use mice in this project. We anticipate the use of approximately 2200 mice over 5 years.

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

Work on developing treatments for epilepsy can involve the generation of models of those diseases in order to test the effects on the nervous system of the disease and whether our treatment effectively reduces symptoms. We make all efforts to reduce

adverse effects, and we undertake careful monitoring so that if and when animals appear distressed or suffering we end the experiments. The adverse effect most likely to occur is post-surgery weight loss. Therefore, we will investigate feeding options to improve this such as high calorie food. Conditions of chronic epilepsy will be generated by injecting chemicals in the brain that overexcite brain cells. The severity of the seizures will be kept to a minimum by carefully controlling how much chemical is injected and should very rarely exceed a moderate severity. Animals will be killed using humane methods before seizures become too severe or if they show adverse effects, such as weight loss.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

These new treatment strategies cannot be tested in humans as they are as of yet unproven to decrease seizures. Therefore, the risk would be too great for patients to directly undergo this treatment. Furthermore, even though the tools that we need to test these strategies are ready in mice, they are not ready yet for humans. It is also not possible to use in vitro experiments as these do not mimic the human version of seizures accurately. Finally, computer models are also not appropriate as there are too many variables that could influence the outcome of the experiments due to the complexity of brain circuits, which we could not possibly factor into a computer model.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

In order to use a minimal number of animals we will use long term recordings, which will allow us to record many seizures in individual animals. In the models we use the majority of seizures do not have a noticeable effect on the behaviour of the animals, but are instead only registered through electrographical recording. A high number of seizures allow us to use less animals as a high number of seizures in individual permits for statistical tests to be performed on fewer animals.

A goal of the project is to identify which light stimulation parameters are the most effective at blocking seizures. Since we will record a large number of seizures we will be able to test multiple types of stimuli in single animals.

We will also be mapping neuronal networks with new technologies that allow for a much higher degree of certainty of whether connections are actually present. This is also done through the use of optogenetics as we can record the signals received by specific brain cells when we control the activity of other cells.

Breeding will be managed carefully in order to minimally breed transgenic animals.

Refinement

Explain the choice of animals 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.

Refinement

Seizure severity will be kept to a minimum by carefully dosing the amount of chemicals injected in the brain that produce seizures. For the most part, the seizures that we record in the animals with our electrodes will not spread to other parts of the brain. This means that these seizures will very rarely actually cause a mouse to seize and have an observable seizure.

For our in vivo models of epilepsy and expression of exogenous genes, we endeavour to refine the experimental protocols, we will routinely provide enriched environments (e.g. cardboard castles) for recovering animals, and allow animals to socialise where solitary housing may be stressful. We also consistently seek foods which are most favoured for post-operative animals (e.g. Nutella, peanut butter, apples etc.). Optogenetics and telemetry recordings are also forms of refinement. Optogenetics increases precision of our neuronal manipulations, while telemetry recordings allow for freedom of movement of the animals within their cages.

PROJECT 21

NON-TECHNICAL SUMMARY (NTS)

Project Title	Skin homeostasis and wound healing
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Wound healing involves a series of overlapping, highly organised processes to ensure that the skin heals successfully. When these processes are disrupted, healing is delayed. If this delay is sufficiently severe then it leads to a chronic wound. Chronic wounds are a significant global problem. The incidence of chronic wounds is currently rising because those populations most susceptible, the elderly and diabetic, are rapidly expanding. This puts increasing financial strain on the world's health services. In 2014, the annual NHS spend on wound care was over £2 billion, while in the U.S. an estimated US$25 billion is spent on their treatment. Despite the global economic and social impact of chronic wounds our understanding of delayed healing remains poor. This is particularly true for wound infection. As many as 85% of lower limb amputations are preceded by an infected chronic wound. The high amputation rate highlights the failure of current therapies to treat infected wounds. This failure is partly due to:

1. The increase in antibiotic resistant bacteria.
2. The bacteria protect themselves by forming a biofilm.

Biofilms form when bacteria group together, attach to the wound bed and secrete substances to form a protective coat. Biofilms avoid the body's immune response and are resistant to antibiotics. Clinical evidence suggest that biofilms are a key reason why some chronic wounds do not heal. However, our understanding of how biofilms delay healing and which bacterial species are the most detrimental to healing remains poor.
In addition, the effects of therapies to promote healing (such as antimicrobials) on normal intact skin or uninfected wounds are often unknown. A greater understanding on which processes of wound repair therapies effect is needed to guide clinical practice.

Our objectives are:

1. Develop new clinically relevant models of delayed healing (such as a biofilm infected wound model). These models will provide an essential tool to address objectives 2 and 3.
2. Study the cellular and molecular mechanisms that cause delayed healing.
3. To understand how current and new wound healing therapies affect different aspects of wound repair and normal intact skin.

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 results of this project are intended to: 1. Develop and improve models of poor healing. 2. Increase scientific knowledge of the mechanisms which contribute to poor healing. 3. To improve treatment regimens by better understanding how treatments affect healing.

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

3000 mice for 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?

This study is designed to better understand delayed wound healing. All wounds will be made under anaesthetic and with post-operative pain relief and monitoring. Anaesthetised mice (wild-type, ovariectomised and diabetic) will receive small (10 mm incision or 6 mm diameter excision or less) wounds on their skin so that we can understand the processes involved in wound healing. After surgery, mice will be provided with pain relief and monitored closely for any signs of distress. Distress in mice after this type of surgery is very rare; however, if there is any indication of suffering we will seek veterinary advice. In some cases bacteria/fungi will be applied to the wound to represent wound infection. Wound infection is not expected to become systemic but is likely to delay healing. Animals will be monitored closely for illness and advice sort where necessary. We will apply different factors to the wounds that we believe will enhance healing, including antimicrobial agents, in order to understand how these treatments affect different aspects of repair. Animals under any procedure will be monitored daily and appropriate action will be taken for any animal presenting with any obvious stress or discomfort. If there is any indication of suffering animals will be culled. All animals will be culled by a schedule 1 method at the end. The expected severity levels: • Application of therapies on intact skin: Mild • Wounding procedures and ovariectomy: Moderate.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We have expertise in the use of sophisticated cell culture (*in vitro)* and whole pig skin culture *(ex vivo)* models, which we already use to examine skin biology and wound healing. However, these models are not able to fully replicate the complex interactions *in vivo*, such as the inflammatory response. Thus, while we cannot eliminate the requirement for animal use, the *in vitro* and *ex vivo* models will be used

to guide development of animal models and to understand which processes of repair are affected by wound therapies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use cell culture (*in vitro)* and whole pig skin culture *(ex vivo)* assays where possible to examine individual processes relevant to wound repair and will use these prior to *in vivo* work. To plan for our animal work, we have consulted a statistician to establish the minimum number of animals required for each study. Also, where possible, we will use two wounds per animal to reduce the number of animals required.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the most widely used species for skin research. We have many years of experience in this field and hence models have already been extensively optimised. For example, we have shown that hair cycle influences the rate of wound healing; in all studies we now assess the stage of the mouse hair cycle prior to wounding and only wound animals when hairs are in a specific hair cycle stage. By performing this step we reduce variability and group sizes within experiments. In addition, we have statistically analysed the variation in healing between two wounds on the same mouse compared to wounds from different mice. This has allowed us to refine our experiments to use a wound (not animal) as a biological replicate, therefore reducing the number of animals required. We are also aware that the time of day when mice are wounded may affect healing; therefore we will plan to perform all studies at the same time of day to minimise variation in data.

PROJECT 22

NON-TECHNICAL SUMMARY (NTS)

Project Title	Investigation of host responses to virus infection.
Key Words	Virus infection, vaccine, tropism, hepatitis C virus, Zika virus
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Viruses frequently cause disease in humans and animals. Most viral diseases have no known cure. Furthermore, for many viruses there are no effective vaccines available. The development of effective treatment and prevention requires prior knowledge of how the virus interacts with the host, how various factors (particularly those of the virus and the host) help sustain and spread infection in the host, and how they contribute to pathogenesis. The viruses to be studied in this proposal are hepatitis C virus (HCV) and Zika virus (ZIKV). HCV is a major cause of liver disease in humans. A majority of infected people are left with a long-term chronic infection, which can progress to cirrhosis of the liver often leading to liver failure, or hepatocellular carcinoma. ZIKV infection causes a congenital condition associated with incomplete brain development (microcephaly) in newborns and the neurological condition (Guillane-Barre syndrome or GBS) in adults. It is likely that it causes other, as yet unidentified, neurological syndromes. To date, very little is known about how ZIKV enters the nervous system, which cell types it infects, what are the consequences for the infected cells and whether the neurological symptoms are due to direct viral infection or post-infectious immune responses. Our overall aims are to (1) test and evaluate novel vaccine candidates (generated in-house) against HCV and ZIKV, (2) infect mice with virus to investigate virus tropism (i.e. the ability of the type of host tissues and cells to support virus growth) and pathology, and (3) generate mouse monoclonal antibodies of interest. Antibodies specifically reacting to the relevant viral and host proteins are extremely important tools in studies investigating their functions and the role they play in viral and cellular processes that contribute to pathogenesis

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)?

Currently, there are no vaccines available against HCV and ZIKV. These viruses are a major global health problem. The vaccines targeting these that we propose to develop would be key tools for preventing primary infection by these viruses and for their eventual global eradication. The proposed project will allow us to validate the pre-clinical potential and efficacy of our vaccine candidates in mice. We then intend to take the promising vaccine candidates forward for large-scale production with a view to taking them into clinical trials. We hope to undertake the subsequent late stage vaccine development through partnerships/collaboration with appropriate pharmaceutical company. The determination of how Zika virus enters and disseminates within nervous system, cell-type tropism, the consequences of direct viral infection and whether neurological symptoms reflect post-infectious immune responses will inform the development of preventative and therapeutic measures.

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

We anticipate using 2880 mice in total over the 5 year duration of this project.

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?

All procedures to be carried out are associated with mild or moderate severity rating. Mice undergoing all procedures will be monitored carefully and regularly by the NVS to minimise distress and suffering. At the end of all procedures the animals will be euthanised and tissue will be harvested for analysis. Analysis will be done by a variety of methods including flow cytometry and quantification of cell types, immunofluorescence microscopy and image capture and cell quantification, ELISA, electron microscopy.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The immunogens being developed for vaccine purposes need to be evaluated in a physiologically relevant model for their ability to elicit the desired immune response and confer protection from infection. This can only be done in a living animal. Similarly pathogenesis studies we propose require complex multicellular environment which can only be mimicked in an animal model. For antibody production, the recombinant display based technologies are possible alternatives. However, because the antibody-encoding genes originate from naïve (i.e. non-immunised) sources, they are not suitable for the production of the antibodies with desired specificity and affinity to the antigen of interest. These properties are crucial for their use in studies investigating protein structure and function.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We propose to restrict the number of animals to a minimum required to provide statistically significant analysis. Specifically, we will use the resource equation described by Mead (1988, The design of experiments. Cambridge, New York: Cambridge University Press. 620 p.) as a tool to determine animal group size as indicated in the application above. Power calculations will be done following preliminary animal infection experiments to determine minimum n values. In keeping

with the principles of Reduction, we propose to use the best responder group of animals vaccinated with ZIKV antigens (from Protocol 1) in challenge experiments with live ZIKV (Protocol 2). This means actual reduction in numbers as it will obviate need to repeat the desired procedures using a new group of animals

Refinement

Explain the choice of animals 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.

Refinement

Mice have traditionally been the most successful animal models for primary evaluation of immunogens and for generating monoclonal antibodies. The proposed immunization protocols are designed to cause least pain and suffering. They are also the least sentient animals that are available with the necessary genetic modifications and because their nervous systems are sufficiently similar to that of humans to be informative.

All animals will be monitored daily and will be humanely killed if necessary, following veterinary advice.

PROJECT 23

NON-TECHNICAL SUMMARY (NTS)

Project Title	Recombinant Vaccines for Infectious Diseases
Key Words	vaccines, infectious diseases
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Vaccines are amongst the most cost-effective tools in reducing the burden of infectious diseases. In the past century, widespread vaccination campaigns led to the eradication of smallpox in most parts of the world. However infectious diseases continue to cause significant morbidity and mortality. The WHO estimates that 23% of deaths worldwide are due to infectious diseases, with the highest burden in children under 5, where 64% of deaths are infectious-disease related (*World Health Statistic 2015*). Sadly the majority of these deaths continue to occur in developing countries. Malaria alone caused almost ½ million deaths in 2015, with the highest burden of diseases in children under 5 in sub-Saharan Africa where it accounts for 67% of all malaria related deaths (*WHO 2015 Malaria Report*). In addition to the known big killers (malaria, tuberculosis, Influenza and HIV), emergence and re-emergence of dangerous pathogens have been identified as a serious threat to human health.

The purpose of this project is to develop vaccines against malaria, tuberculosis, Influenza and emerging/re-emerging/neglected diseases. The work is divided into 4 stages

1) Develop new vaccines and vaccination regimens to enhance the immune response

2) Test the efficacy of these vaccines against parasite, bacterial or viral challenge

3) Identify new vaccine antigens

4) Understand the kinetics and mechanism in response to vaccination and infection

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 benefits of this work include 1) The development of vaccines against new emerging pathogens and major diseases traditionally viewed as very difficult targets. 2) Improvement to vaccination regimens to induce better and more robust immune responses 3) Increased knowledge about the immune response to vaccination and ways to improve immunity induced by these vaccines 4) Improved understanding of the immune response to infection by these pathogens (malaria, tuberculosis and Influenza) which will positively impact on future vaccine design. As the viral vector and recombinant vaccine technology investigated is broadly applicable to development of vaccines for other diseases requiring robust cellular and/or humoral immunity, this could lead to the development of vaccines against other communicable and non-communicable diseases for both human and veterinary

medicine. In addition, better vaccine design can only be achieved through improved understanding of the immune response to infection, as this can identify the type of immune response required and potential antigenic targets.

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

We will use a maximum of 31350 mice and 100 rats over 5 years. On average we have 20 active PIL holders per year with each using approximately 300 mice per year

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

The majority of mice will be purchased from a commercial supplier and be vaccinated with a vaccine intramuscularly on two separate occasions. Mice are typically killed 2 weeks after the last vaccination and the immune response measured in an in vitro assays. On some occasions it will be necessary to challenge mice with malaria, tuberculosis or Influenza to demonstrate the efficacy of our vaccines. These mice may get sick as a result of the infectious challenge, however the disease course is well documented and mice are killed when moderate symptoms of disease develop. For the majority of mice challenged with an infectious organism, the scientific endpoint of the experiment is reached before mice show signs of systemic disease (liver-stage malaria challenge and tuberculosis). All mice are killed at the end of each experiment. To maintain our colony of mosquitoes and to provide a source of malaria parasites for challenge experiments, it will also be necessary to allow mosquitoes to feed on anaesthetised mice. To investigate the role of CD8+ T cells in mediating protection from malaria at the single cell level, we will perform multi-photon live imaging experiments. Whole mouse imaging will also be used to measure malaria parasite burden in the liver or vaccine distribution/longevity. As one main of the main hurdles in the development of vaccines against human diseases in the inability of the species that infect humans to infect rodent models (particularly malaria species). We will use humanised mouse models that can be infected with malaria in experiments to identify new malaria antigen targets.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The immune response to vaccination and infectious disease involves multiple, complex systems interacting in a physiological environment often involving

antibodies, T cells and cells of the innate system and therefore cannot be replicated in tissue culture. For pre-erythrocytic malaria there is currently no *in vitro* predictors of *in vivo* efficacy, although a number of exploratory *in vitro* assays are under assessment in our laboratory that might assist towards this goal. We will continue to investigate *in vitro* assays and *in vitro* organ culture systems.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Throughout the project we will employ several strategies to minimise the number of animals used:

1) Prior to experimental work statistical power calculations will be performed allowing use of the smallest number of animals needed to provide satisfactory analysis of the data. We will continuously evaluate and update our statistical approaches and group sizes.

2) Past experience enables selection of experimental time points that maximise the amount of data while using the minimum number of time points.

3) Many experiments necessitate the inclusion of control groups to enable the comparison of new vaccines to a gold standard or most-promising vaccine to date. To minimise the repeated use of control groups, as many test conditions as possible will be included in an experiment. However the overall size of the experiment will be limited so as not to compromise the scientific integrity of the experiment.

4) Where possible the data from each individual animal will be maximised through the collection of multiple tissue samples at endpoints and/or sequential sampling from the same animals across a time course, eg blood sampling or whole imaging during sporozoite challenge.

5) The number of excess mice generated through breeding will be minimised by constant and careful monitoring of breeding programs.

Refinement

Explain the choice of animals 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.

Refinement

We have chosen mice for these studies since they are the most characterised species for detailed immunological analysis. Mice have proved to be excellent

indicators of immunogenicity enabling the clear assessment of novel vaccines and vaccination regimens for improvements. For isolation of primary hepatocyte we will also use rats that are larger and provides higher numbers of viable cells.

To minimize suffering we only use well established disease models and the majority of mice are killed before systemic disease develops. Monitoring is tailored for each disease and is increased around the peak time of illness to ensure animal suffering is minimised. During an Influenza challenge when protected mice lose weigh before overcoming the infection and putting weight back on, water and food (often as mash) is placed on the cage floor to support animals during their most vulnerable period.

We continue to investigate ways to refine our techniques to minimize animal suffering. Our previous experience ensures we can reduce the number of blood samples to only the key timepoints post-vaccination. As the primary focus of our program is clinical deployment, we primarily use adjuvants that have been approved for use in humans and have minimal side-effects.

PROJECT 24

NON-TECHNICAL SUMMARY (NTS)

Project Title	Antibody-based Intervention for Disease Protection
Key Words	Vaccines, Antibodies, Malaria, Therapeutics, Disease
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Antibody responses underpinned the great success stories of 20th century vaccinology. Indeed, vaccines are the pre-eminent cost-effective tool for reducing the burden of infectious disease. In the past century, widespread vaccination campaigns led to the eradication of smallpox in humans and rinderpest in cattle, with polio also on the brink. However, despite these great achievements, many difficult diseases remain against which highly effective antibody-mediated immunity fails to arise or for which effective vaccines have simply not been developed. In addition to the known 'big killers' (malaria, tuberculosis and HIV), emergence and re-emergence of outbreak pathogens have been identified as a serious threat to human health, exemplified by the 2015 Ebola virus epidemic; and new approaches are needed to tackle disease in the ageing population.

In parallel to these advances in vaccinology, antibodies have also made a huge impact in the field of biologics. Monoclonal antibodies (mAbs) are now essential treatments for inflammatory and autoimmune conditions, as well as cancer. With the deployment of ZMapp as a frontline investigational treatment for Ebola virus, there is renewed interest in the rational improvement of these antibody-based therapeutics for emerging and outbreak pathogens.

This work will be divided into two major stages: the development of improved vaccines including new-generation technologies such as virus-like particles (VLPs) and vaccine adjuvants (Objective 1) and the development of new antibody-based drugs (Objective 3). In doing so, a large part of this work will focus on malaria, and so we also aim to better understand the mechanisms of malaria immunity and control (Objective 2), so that we can apply this knowledge into the design of further improved intervention strategies.

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 potential benefits of this work include 1. The development of new vaccines, adjuvants, VLPs, or antibody-like drugs against major difficult disease areas including malaria; new emerging pathogens such as the Ebola and Zika viruses; and non-communicable diseases such as disorders of red blood cells. 2. Improved vaccination strategies to achieve effective immunity, prophylaxis or therapy. 3. Increased knowledge about the immune response to vaccination and ways to improve immunity induced by these vaccines. 4. Improved understanding of the immune response to malaria infection which will positively impact on future vaccine design. Given our technologies are broadly applicable, these could lead to the

development of effective interventions against other communicable and non-communicable diseases for both human and veterinary medicine.

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

We will use a maximum of 21,000 mice over 5 years.

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

The majority of mice will be vaccinated with a vaccine formulation intramuscularly on three separate occasions. Mice are typically killed 2 weeks after the last vaccination to measure immune responses or to isolate antibodies from cells or serum. Most of these procedures will be mild in severity and the mice will receive appropriate anaesthetic during procedures. Sometimes mice may experience short-lived reactogenicity following vaccination, with mild symptoms just like humans. Some mice may be allowed to age to monitor responses over a prolonged period of time, and therefore may develop adverse effects related to the natural ageing process. Mice will be closely monitored in case these occur. On some occasions it will be necessary to challenge mice with malaria to demonstrate the efficacy of our vaccines or antibodies, or to investigate the mechanisms that underlie protection against disease. These mice may get sick as a result of the infectious challenge, however the disease course is well documented and mice are killed when moderate symptoms of disease develop. All mice are killed at the end of each experiment.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The immune response to vaccination and infectious disease involves multiple, complex systems interacting in a physiological environment often involving antibodies, T cells and innate cells and therefore cannot be replicated in tissue culture. It is also not possible to generate complex antibody responses outside a functioning immune system in an animal. We have developed a number of new *in vitro* assays that have allowed us to replace the need for animals to test the function of the antibodies that we induce by vaccination.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Throughout the project we will employ several strategies to minimise the number of animals used:

1. Prior to experimental work statistical power calculations will be performed allowing use of the smallest number of animals needed to provide satisfactory analysis of the data. We will continuously evaluate and update our statistical approaches and group sizes.

2. Past experience enables selection of experimental time-points that maximise the amount of data while using the minimum number of time-points.

3. Many experiments necessitate the inclusion of control groups to enable the comparison of new vaccines to a gold standard or most-promising vaccine to date. To minimise the repeated use of control groups, as many test conditions as possible will be included in an experiment. However the overall size of experiments will be limited as far as may be possible without compromising their scientific integrity.

4. Where possible the data from each individual animal will be maximised through the collection of multiple tissue samples at endpoints and/or sequential sampling from the same animals across a timecourse, e.g. blood sampling during a vaccination timecourse.

5. The number of excess mice generated through breeding will be minimised by constant and careful monitoring of breeding programmes.

Refinement

Explain the choice of animals 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.

Refinement

We have chosen mice for these studies since they are the most characterised species for detailed immunological analysis. Mice have proved to be excellent indicators of immunogenicity enabling the clear assessment of novel vaccines and vaccination regimens for improvements. To minimise suffering we only use well established disease models and the majority of mice are killed before systemic disease develops. Monitoring is increased around the peak time of illness to ensure animal suffering is minimised during a malaria infection study. Also, in an efficacy study, if 50% of the control mice reach the humane endpoint, any other mouse whose parasitaemia is increasing at the identical rate, will be killed to minimise suffering in other animals.

We continue to investigate ways to refine our techniques to minimise animal suffering. Our previous experience ensures we can reduce the number of blood samples to only the key time-points post-vaccination. As the primary focus of our programme is clinical deployment, we primarily use adjuvants that have been approved for use in humans and have minimal side-effects.

PROJECT 25

NON-TECHNICAL SUMMARY (NTS)

Project Title	Brain circuit dynamics in health and disease
Key Words	Neuroscience, Neurophysiology, Neural circuits, Schizophrenia, Dementia
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Neuroscience has made great strides in understanding how individual parts of the brain function, but much less is known about how different parts of the brain co-ordinate their activity. The aim of this project is discover the ‘wiring diagram’ of how different brain regions are connected, and to learn how these connections change in diseases such as dementia and schizophrenia. Our main focus is on parts of the brain that are essential for making decisions, storing memories and navigating through space, as the connections between these parts of the brain are disrupted in a number of different diseases affecting memory, such as Alzheimer’s disease. We will study both anatomical changes (to study whether one region make unusual connections with another in schizophrenia, or whether specific links between brain regions die off in dementia) and functional changes (changes in the electrical activity of the brain, or changes in animal behaviour) caused by these conditions.

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 regions of the brain that we study are essential for memory and for carrying out tasks such as navigation and making decisions. These processes are disrupted in many different brain diseases, including dementia and schizophrenia. By studying how the same parts of the brain change in different diseases, we hope to also gain insights about how these areas function and communicate in healthy systems. The main benefits from this project will therefore be twofold: increased understanding of how our brains work when we are healthy, and the discovery of new processes that could potentially be targeted to develop new treatments for a number of diseases affecting the brain.

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

The majority of our experiments will use mice (up to 5000 over 5 years), although a small number of experiments will use rats (up to 400 over 5 years). We will primarily use mice because a number of advanced tools for studying brain function have been developed in genetically-altered mice, and because well-established models of both Alzheimer's disease and schizophrenia have been developed in genetically-altered mice. Rats are more intelligent than mice and are larger, so we may carry out a small number of experiments in rats when these attributes make them more likely to be able to successfully complete an experiment.

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

Animals that are being used as a model for schizophrenia or dementia usually show subtle changes in behaviour or brain activity and typically do not experience suffering from the aspect of the disorder that is being modelled. These disease models normally use genetically-altered animals, but in some cases we will treat the animals with phencyclidine (PCP) daily for several days to induce a psychosis-like state. PCP is a controlled drug that is used recreationally by humans, and its use is a well-established model of psychosis in animals. Apart from mild stress of being handled for injection, animals tend not to suffer adverse effects at the doses needed to see changes relevant to this project. Many of our experiments will require surgery where we can use viruses to insert genes into various parts of the animal brain to allow us to change the way that they function, or to better study how different parts of the brain are connected. In these cases, animals will undergo surgery lasting around an hour, where we make a very small window in the skull to insert a small needle to inject the virus into the brain. We will then seal up the skull and allow the animals to recover. Typically, animals experience some pain after the surgery, but this can be easily controlled with medication, under the guidance of a vet. Animals typically recover within a few days, and are housed normally for several weeks until they are killed under deep anaesthesia so that the brain can then be removed for further study. In a smaller group of experiments, we will also implant devices into the animals' brain to allow us to record electrical activity while they carry out behaviours such as exploring a maze. During these surgeries (typically lasting 2 hours), animals will be deeply anaesthetised and small devices (weighing a few grams) will be implanted into their brains and secured in place using dental cement. At least a week after surgery, animals will undergo behavioural experiments, during which will be connected to recording apparatus via a wire, which can cause some initial distress. Animals quickly get used to this type of experiment, and become comfortable enough to fall asleep during the experiments. Before each behavioural experiment, animals may receive an injection of a drug designed to increase or decrease activity in a specific part of the brain to allow us to better understand how that part of the brain is needed to carry out the task. At the end of these experiments, animals will be killed under deep anaesthesia to allow us to remove the brain and study anatomical changes.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Our research aims to discover how different regions of the brain are connected in both health and disease. Very little is known about these connections so the only way to learn about them is to directly study the brains of animals. Our overall goal is

to be able to relate our findings to human brains and human diseases. We are interested in the complex connections linking different parts of the brain, so need to study an animal that has analogous regions to those in humans. As such, mammalian models are essential for this work as non-protected animals such as fruit flies and nematode worms do not possess sufficiently complex brains. Much of our work will be carried out in rodents, as they have the same brain regions as humans, albeit in a simpler form. Furthermore, numerous genetic tools for studying the function of brain circuitry exist in genetically-altered mice, so using mice will enable us to take advantage of these tools.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Whenever possible, we will use a factorial experimental design, which involves testing more than one condition at a time, to allow more robust statistical inferences to be made from as few animals as possible. For circuit mapping experiments involving recovery surgery, we will aim to maximise the data we can obtain from each individual by using as both sides of the brain whenever possible. A number of our behavioural experiments are designed to observe animals carrying out natural behaviours (e.g. exploring a new environment) so to minimise the numbers of animals used, we will use first use animals in this kind of behavioural experiment before then training them to carry out a different task (such as working to earn food rewards in maze).

Refinement

Explain the choice of animals 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.

Refinement

The aim of our research is to make inferences about human brain physiology through use of animal models. Mice are one of most commonly used mammalian models in research, and genetically-modified mice allow us to target specific types of brain cell, so mice are the most appropriate mode for our research as they allow us to gain the largest amount of information from the smallest possible number of mice. Genetically-altered animals will be used wherever possible to model dementia or schizophrenia. The models that we will use are well-validated and are known to faithfully recapitulate specific aspects of human disease, so represent the most refined way of studying these disorders.

Animals will be housed to the highest welfare standards possible, and all surgical procedures will be carried out using appropriate post-operative analgesia and care to minimise the suffering and stress. After surgery (injections of viruses into the brain or implantation of recording devices), animals will be monitored closely for 1 week to ensure that they are recovering appropriately and any animals failing to show adequate improvement will be killed humanely

All behavioural tests will be carried out with as little stress to the animals as possible; indeed, many of the behavioural tests promote behaviours that rodents would display in the wild so could be considered a form of environmental enrichment. Some behavioural tests require animals to work for food rewards so in these cases, they will have access to food restricted for a maximum of 16 hours per day. This degree of food restriction is considered mild and animals that undergo this type of restriction have been shown to live longer and be healthier than mice allowed to eat whenever they want.

PROJECT 26

NON-TECHNICAL SUMMARY (NTS)

Project Title	Epigenetic control of mammalian development and genome function
Key Words	Epigenetic, imprinting, genome, mechanisms, development
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

Our genes are the DNA code that is the blueprint for life. Epigenetics is a layer of chemical marks that sits on top of our DNA that make our different cells (which all contain the same DNA and genes) behave in a specific way. For example, it makes liver cells behave like liver cells and brain cells behave like brain cells etc. Genes are present in two copies. One copy is inherited from the mother and one copy is inherited from the father. Some genes are epigenetically marked whereby only one copy is switched on depending on whether it is inherited from the mother or the father. These important processes contribute to mammalian development and their failure can influence human health.

The aim of the research in our laboratory is to understand the function of epigenetic marks and the DNA and genes that they regulate. We want to understand how they control growth and development of the baby, the placenta and the brain and how these genes regulate metabolic processes associated with diseases such as obesity and diabetes and how their altered regulation causes disease and aging. We also aim to understand epigenetic marks controlling the amount of gene product present in the body which when altered can cause cancer and other diseases. From our studies, we have discovered that the amounts of some genes is also involved in tissue regeneration. From these results we can investigate the regeneration process in our genetically altered animal models.

This project will generate genetically altered mice in which these processes are perturbed and compare them with normal mice. We wish to understand important epigenetic marks and how their control contributes to all stages of development. To help us to understand these mechanisms we will study the effects of these alterations on the well-being of the mice and their offspring. We will also study environmental factors which affect the epigenetic marks such as diet and aging. To complement our studies we will also use zebrafish, which have many of the same genes and pathways as mice but are simpler to study.

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 findings from our research will provide more information on how epigenetic marks contribute to normal processes in all stages of development from pre-implantation to perinatal to the adult. This includes the regeneration process which can occur in our cells following tissue damage. This information will also help us to understand how these processes are perturbed causing disease states such as

obesity, diabetes and cancer. This in turn will help in the development of therapies in tissue regeneration and to target epigenetic changes that cause disease.

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

We will use two animal models to conduct our experiments; mice and zebrafish. Over a period of 5 years our projected use of mice is 18700 and 20500 zebra fish (larvae, juveniles and adults). These include a number of protocols and as we continue to work on our reduction and refinement we believe that we will work below these numbers.

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

We have protocols that are classed as mild and moderate severity. However, many of our animals actually only experience a mild or less than mild severity level. New genetically altered mice strains that we generate are closely monitored for adverse effects. An adverse effect that they may encounter is restricted growth during development in the mother, which is sometimes recoverable after birth as the mouse gets older. We also study the regeneration of cells in animals. In mice we administer a substance into the muscle to cause cell damage. This is done using the lowest dose possible to achieve an effect and is performed under anaesthetic. The mice may only suffer from mild inflammation and they are expected to make a full recovery. It is this recovery process that we will investigate.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We cannot conduct all our experiments on cultured cells as we are assessing the health of the whole organism. Epigenetic states *in vitro* are also very different to their natural states *in vivo*. We are studying mechanisms and pathways in the developing organism and we therefore need to look at different time points during development. As cultured cells are exact copies of each other this means that changes occurring in developing cells cannot be seen. However we use in house and public databases and reanalyse existing data instead of rerunning experiments wherever possible. We perform initial experiments in cultured cells to test some of our hypothesis and choose important genes from these results before moving into animal experiments.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have been working with mice for over 25 years and we have expertise in statistical analysis and optimal experimental design to determine the minimum number of animals needed to achieve robust, meaningful data. We collaborate with other groups, share mice tissues and data. We statistically analyse mouse numbers for use in experiments, plan experiments responsibly and communicate between lab members to make the best use of our resources. We use databases to find candidates genes and conduct studies first in cell lines whenever possible. We are collaborating with local colleagues who are experts in theoretical and mathematical modelling who will provide added value and novel insights to the animal work. Where appropriate we use control litter mates or use control tissue from the same animal. Where appropriate we randomly assign animals to control and test groups and analyse samples blind to avoid bias. Breeding colonies are generally kept small following good colony management strategies.

Refinement

Explain the choice of animals 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.

Refinement

The mouse is the best model for these studies because a catalogue of all the genes in the mouse exists and there are well-established procedures that are not harmful for the animals which can be used to mutate the genes or change their regulation. Many of the genes found in mice are found in humans too. In addition we can breed the mice selectively and follow the effects in their offspring for multiple generations.

We keep up-to-date with new technologies and developments that allow us to refine our experiments. We continuously monitor our animals and work closely with the vet and the staff in the animal unit to ensure the animals reach a humane end point and receive the best welfare possible.

PROJECT 27

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Modulating Radiosensitivity in Tumour and Normal Tissues
Key Words	Cancer, Radiation, Microenvironment, Immune cells
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Patients with cancer frequently receive radiation therapy as part of their treatment. While this is often helpful, unfortunately in many cases the therapy fails to be curative. We intend to develop a better understanding of the biology of the tissue response to radiation in order to develop means of improving the outcome in patients. Based on our understanding of the response to radiation we intend to perform pre-clinical tests of strategies to improve therapeutic outcomes of radiation.

Very little is known about how some of the components of a tumour respond to radiation including the tumour blood vessels and the immune cells. Further, radiation is delivered to cancers in man in multiple small doses daily over 3-6 weeks, whereas most experimental studies in mice are based on a single large dose. Thus in these studies we intend to determine, using mouse models of cancer, what happens to tumours, the cancer cells, the blood vessels and the immune cells, in response to radiation delivered both as a single dose and in the multiple fractions typical of patient treatment. We intend to ask whether perturbations of the vascular or the immune response to radiation affect the ability of radiation to eradicate cancer cells. The design of the preclinical work would be intended to ask whether the strategies employed would be applicable to human tumours.

It is known that decreased delivery of oxygen to a tumour, hypoxia, results in a reduced cure from radiation because hypoxic cells are more resistant to radiation. Based on our observations of the changes in hypoxia and the mechanisms underlying them, we will test whether hypoxia can be manipulated in murine tumours and whether these strategies could be used in treatment of human cancer. Since vascular efficiency is linked to oxygen delivery and hypoxia the evaluation of hypoxia and effects on vasculature form complementary approaches.

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

We expect that better scientific understanding of how radiation therapy affects tumours will be obtained. Thus the science of understanding how organisms respond to tissue damage, especially induced by radiation and thus to cancer therapy will be advanced. We also expect that new clinical strategies for radiation therapy will emerge.

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

All of our work will be performed in mice. We expect to use up to 7,600 mice over 5 years.

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

The core of the experimental protocols involve the induction of tumours in mice and the effects of their irradiation, asking whether pharmacologic manipulations can be used to improve the ability of the radiation to delay the growth of the tumour. Thus the mice will bear tumours, but we do not expect this to cause pain or discomfort. The mice will receive injections of the pharmaceutical agents and anaesthesia during imaging of the tumours. We expect to use pharmaceutical agents that have minimal side effects. The tumours will receive radiation but the doses and scheduling used usually cause minimal systemic effects or discomfort. The animals will be closely monitored and either treated or humanely killed if they become unwell. All mice will be humanely killed at the end of the study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Currently it is not possible to simulate cancer cells in the environment of a tumour, which includes not only the cancer cells but also blood vessels, many different types of immune cells, fibroblasts and extracellular molecules. While several components can be mixed in tissue culture, this type of experiment cannot include many of the key constituents. Once specific interactions are identified, some of their characteristics can be simulated in tissue culture. In some cases preliminary studies such as screens for drugs that might be predicted to alter particular components of the tumour will be employed to identify candidates to test in vivo. But in the end, only the in vivo experiments have the capacity to put the multiple features into play and the capacity for testing clinical strategies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We use statistical calculations based on prior experience to minimize numbers that would be expected to give rise to an experiment that has statistical power. We also attempt to obtain the maximal amount of information from every experiment to reduce numbers. Finally, by using imaging techniques, we can follow the same animal over time, enhancing statistical power and reducing the need for additional animals at each time point. Pilot experiments also inform as to the conditions and timing that should lead to the best results from the fewest animals.

Refinement

Explain the choice of animals 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.

Refinement

Currently murine models are the most widely used to ask about response of cancers to radiation therapy. Because our goal is to understand the response of the host to the irradiated tumour, tissue culture will not substitute for the in vivo experiments although they can provide mechanistic understanding and identify initial candidates.

We make extensive use of imaging approaches to maximise the amount of information we can obtain from each animal. This may be aided by the use of genetically altered animals in which cells or molecules of interest appear fluorescent. One way we use such mice is to replace the skin overlying a tumour with a glass 'window', through which we can microscopically visualise the behaviour of blood vessels, tumour cells and host cells during tumour growth and following treatment.

To minimize welfare costs to the animals, we monitor them closely. We perform pilot experiments to maximize efficacy of the timing and dosing in the experiments. Pilot experiments are also performed on small numbers of mice to study the growth and behaviour of new tumour systems so that action points, monitoring schemes and humane end points can be defined before larger experiments are commenced. Another approach to decrease welfare costs is the use of aseptic techniques in our surgical procedures, which minimizes risks of post-operative infection.

We will use anaesthesia and analgesia where possible, to reduce temporary discomfort or stress during a procedure. Further, in some cases anaesthesia can be avoided by the use of MRI or ultrasound imaging with hand restraint.

Tumours are grown superficially, under the skin or in breast tissue, so that when radiation is given, the tumour alone can be targeted and surrounding healthy tissues can be shielded easily. Additionally, we use a small animal radiation research platform (SARRP) with the same aim. Appropriate doses will minimize secondary effects of the radiation.

PROJECT 28

NON-TECHNICAL SUMMARY (NTS)

Project Title	Monoclonal Antibody Development
Key Words	Monoclonal Antibody
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
Yes	(g) forensic inquiries.

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

Antibodies are proteins produced by the body to defend against disease by binding to harmful molecules and bacteria. They can be isolated and have become essential tools in a wide variety of biological assays including the diagnosis of disease, roadside testing and the detection of harmful substances.

We propose to raise antibodies to various pesticides, drugs of abuse, poisons and other harmful contaminants. Our final patented rapid assays can then be used for screening for harmful contaminants.

The main rapid assay that we plan to develop is a dipstick. These types of assays are used every day, the best example is the pregnancy test. They use antibodies raised against the hormones that are increased during pregnancy and if present a blue line is seen.

Once the antibody has been raised. Spleen cells are removed and manipulated in culture and fused with a myeloma cell line to produce a cell that will grow in tissue culture and produce antibodies. If successful will result in a permanent source of antibody without the need for further animals.

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)?

Two important areas in which we plan to use them include:- Driving under the influence of drugs is an issue of growing concern and has become more prominent than drunk driving in western countries. By producing antibodies to these drugs we can develop dipstick assays that can be used by Police Officers and customs officials to check for the presence of the drugs. When fully developed these assays could be used at the roadside in the same way as the current Breathalyzer. Herbicides and pesticides, whilst beneficial for crop yields, can be very toxic if they are over used and enter our food chains and water courses. Antibodies raised against these pesticides and herbicides can also be incorporated into dipstick assays. They can then be used to check streams flowing into reservoirs for contamination, or contaminants in raw materials prior to food production.

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

For our purposes we use, female mice, hamsters or rats usually 6-8 weeks old. Females across all species are better antibody producers. Each project would

normally require either the use of 5 mice or 2 rats. The projected number for the duration of a five year program would be 1,000 mice, 250 rats.

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

The protocol allows an initial immunization in Freund's Complete Adjuvant followed by up to 10 boosts in Freund's Incomplete Adjuvant with a test bleed after 4 boosts. This will enable us to see how the immunization is going and if the antibodies are present and in high enough numbers (high titer) The mouse will be humanely killed and we will remove the spleen and perform the tissue culture. With the number of boosts being a possible 10 I have classed this as a moderate severity and although most of the Projects will come to a conclusion after 4 boosts there is still the potential that some will need more than 4 boosts. Discrete lumps may from time to time develop at the site of injection (due to the use of Adjuvants). Although we have not experienced this during the life of the previous license. We still minimise the occurrence by: 1) Restricting the use of Freund's Complete Adjuvant to the primary injection only 2) Distributing the immunogen to at least two sites and not exceeding 0.05ml (0.1ml in total) in each site in mice rats and hamsters. In the event of ulceration or an abscess occurring, the NVS will be consulted. If there is no improvement within 2 days the animal will be humanely killed. An adjuvant (a chemical to stimulate the immune response) is co-injected with the harmful molecule. Although these harmful molecules can be toxic in the environment they are used in this context in a dose that is non-harmful to the animals. The adjuvant itself, may cause abscess or ulcer formation. Less irritant alternative adjuvants are constantly being sought; these will be employed as they are developed. Hypersensitivity may occur and an animal may suffer an anaphylactic reaction. There was a single incidence of this during the previous project license where the immunized animal died after the final immunization prior to the animal being humanely killed and spleen removed. As with any project concerned with raising antibodies to antigens there may be occasions when this occurs. In our experience this is most commonly associated with administration of the final boost of antigen. This final boost is normally an I.V injection of antigen. The incidence of anaphylaxis (an allergic reaction) can be reduced by giving the animal an injection of "Piriton" (anti histamine) immediately prior to administration of antigen. We have reduced the incidence of this adverse effect further by • Omitting administration of the final boost to animals which have developed a high antibody titer. • Administering the final boost of antigen by subcutaneous injection rather than intravenously. Since the introduction of these changes there has been no further incidence

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Other technologies to make monoclonal antibodies do exist but are still in their infancy e.g. Phage Display these are quite often weak affinities compared to the high affinities we get when we use conventional immunizations, that utilize the animal's immune system to make antibodies to molecules of interest. Until other technologies are able to provide us with antibodies that have the same affinities and can be grown to the required amounts in tissue culture, animals and hybridoma fusion will be used.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Not everyone who is immunized will produce antibodies this is the same when using animals. We have increased the changes of successful outcome by using;

- Females
- Our tailored immunization.
- Increased number of boosts for the more difficult molecules.

All of these mean fewer repeats using new animals.

Refinement

Explain the choice of animals 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.

Refinement

Our work has been refined using experiences gained form previous projects.

- We use the female of the species generally the females of different species are better at producing antibodies.
- Our conjugates are made to insure the best chances of a successful immunization, minimizing the need to repeat with new animals.
- All the materials selected are checked to minimize harm to the animal.
- We do not use harsh chemicals.
- We inject smallest amount possible to minimize lumps, sores etc. Distributing the immunogen to at least two sites and not exceeding 0.05ml (0.1ml in total) in each site in mice rats and hamsters
- All immunizations are carried out by trained animal technicians.
- Our immunization schedule has a test bleeds after the 3rd or 4^{th} boost to assess the antibodies
- All animals with high titers after the 3rd or 4^{th} boost will be humanely killed and their spleens removed for tissue culture

PROJECT 29

NON-TECHNICAL SUMMARY (NTS)

Project Title	Role of intracellular effector mechanisms in immunity to pathogens
Key Words	Virus, immunity, antibodies, infection, TRIM21
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

We are investigating natural immune responses to viral infection and other pathogens and designing antivirals and antimicrobials to augment these processes.

We also interested in neurodegenerative diseases and potential role of TRIM21 in preventing spread of misfolded tau. Finally, we are interested in immune response against gene therapy vectors used to treat 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)?

Infectious disease is the biggest killer of mankind worldwide. Viruses alone kill more than twice as many people each year than cancer. There is therefore a desperate need to understand how pathogens cause disease and the strategies used by our immune system to combat them. We are identifying new anti-pathogen mechanisms and investigating how they work and how they are antagonized by some pathogens. Our ultimate aim is to develop anti-pathogen drugs that will be efficacious in people. Our work will help to understand the biology of AdV vector interaction with immune system and design strategies that potentially open up the use of viral vectors in gene therapy against cancers much more widely than at present.

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

Mice can be used in the development of antiviral strategies and therapeutics. Research programmes are undertaken over 5 years with the expectation to use 16000 mice.

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

The mice will be infected with pathogens such as viruses and bacteria. Only about 10% of the mice will get sick enough to need culling prior to the end of the experiment. Signs such as listlessness, standing in a hunched way, and occasional abnormal breathing will indicate that the mice need to be culled. The remaining 90% of mice show no signs of ill health except weight loss. Weight loss of 20% from the start of the experiment is the endpoint for our experiments. In case of stereotaxic surgery animals are expected to experience only a transient discomfort without lasting harm and should make a rapid recovery from anaesthetic. In the uncommon even that animals failed to do so or exhibit signs of pain, distress or significant ill health they will be culled by Schedule 1 method. In case of external tumour model the majority of the animals are expected to develop external tumours, however mice are not expected to exceed moderate severity. Animals will be culled immediately if

the tumour size reaches 12 mm in any dimension or the total tumour mass reaches 10% of the body weight.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Due to the nature of our research, which is concerned with the response of whole animal immunity to infection, there are no alternatives to an animal model. Where possible we will carry out analogous experiments *in vitro* in culture cells. However, this is not always possible because we cannot recreate the multi-cellular organisational structure that comprises the immune system, *in vitro*.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Breeding programmes are optimised to ensure as little over-breeding as possible. Power analysis will be applied to ensure that only the minimal numbers of animals are used per experiment. Where new protocols are being undertaken pilot studies will be used to inform on the optimum protocol format.

Refinement

Explain the choice of animals 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.

Refinement

Mice are known to share many similarities with the immune response in people, particularly the key anti-viral responses we are studying. We have undertaken in vitro experiments to show that the proteins we work with are highly conserved in mammals.

Genotyping is undertaken almost exclusively from ear biopsies rather than tail biopsy.

Models of disease are chosen to allow the lowest dose of pathogen that allows immune responses in animals to be studied.

PROJECT 30

NON-TECHNICAL SUMMARY (NTS)

Project Title	Provision of blood and tissues
Key Words	Blood, Tissues, Primate
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

To obtain blood samples and bronchioalveolar washes (BAL) from healthy animals housed in their home groups within a breeding colony. Data obtained will be used to inform future or on-going studies by refining assays and defining clinical parameters before any investigative study occurs.

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)?

Expected benefits of this project licence are to enable the provision of non-human primate (NHP) blood on a regular basis and other tissues as they become available to support a wide range of research programmes and to provide materials either for existing diagnostic tests or to support the development of novel in vitro tests. This information is vital to inform the conduct of scientific studies involving NHPs. Tissues and blood from macaques that are of high health status are a rare resource and their similarity to the equivalent human tissue gives added relevance to any tests or research that uses them. In many instances equivalent human tissues cannot be obtained or are rarely available. Such research is aimed at reducing human suffering by understanding disease pathogenesis or producing life-saving vaccines or therapeutics.

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

Adult Macaques: less than 250 over five 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?

Small volumes of blood will be taken and always less than 10% of the circulating blood volume. BAL samples will involve small volumes of isotonic fluid. These procedures will be conducted by experienced licence holders. Experience shows that no adverse effects are expected and the level of severity will be mild. However, animals will be monitored after sampling to ensure that there are no adverse effects. Transient stress due to induction of sedation/anaesthesia may occur during recovery or non-recovery procedures. This will be minimised by use of rapid-acting sedatives or anaesthetics appropriate for the procedure and species based on experience and veterinary advice. Transient stress may occur during recovery from sedation or anaesthesia and this will be minimised by the use of reversing agents where appropriate and by providing a suitable protective environment and level of observation until fully recovered.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The samples generated under this licence will provide blood, tissues and organs with a close similarity to human tissues, that can be used in a wide range of *in vitro* experiments or diagnostic tests. Samples taken will be from animals that are an integral part of a UK breeding colony, with no additional animals being bred specifically to provide them; thus the same number of animals required to form the breeding and issue stock will additionally provide material that will replace the use of other animals for that specific purpose. An example of this is the intention to provide material for cell culture, tissue and organ culture, thus allowing the generation of authenticated primary or immortalised cell lines for distribution for use in medical science and healthcare laboratories.

There are no alternatives to using NHPs for the provision of this tissue. It is anticipated that by being able to provide high quality tissue to appropriate research teams; we can create a resource that replaces the requirement for additional animals (e.g. control groups) to be used.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Samples will be taken only from animals that form part of a breeding group or stock for issue to projects and tissues will only be taken from animals that require culling for colony management or health-associated reasons. Every effort is made to match breeding output with experimental demand for animals and numbers of animals produced will not be increased to solely match a specific demand for blood or tissue. It is envisaged that the use of samples from this colony will reduce the need for projects to acquire additional animals to provide control or naïve samples within their studies.

Refinement

Explain the choice of animals 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.

Refinement

The animal species used will be selected as the most relevant for future or on-going pre-clinical studies evaluating vaccine or therapeutic efficacy. This represents a refinement for the animal by allowing it to remain in the accustomed environment of the colony within an existing established social group rather than under more restrictive experimental conditions. The samples taken will also allow refinement of assays to give the most relevant data during efficacy studies

PROJECT 31

NON-TECHNICAL SUMMARY (NTS)

Project Title	Regulation of Eukaryote DNA replication and maintenance of genome stability
Key Words	Xenopus, DNA Replication, Cell Division, DNA Damage, DNA Repair
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:
No	(I) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall aim of this work is to gain greater understanding into the way cells respond to DNA damage and DNA replication stress and how these responses work to regulate DNA synthesis with cell division and prevent genome instability which is a characteristic of most types of cancer 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 work supported by this project is basic biological research so the immediate benefit will be the advancement of scientific knowledge regarding the ways that DNA Replication and DNA Damage Response pathways are regulated. This information will provide further insight into the ways DNA Replication Stress develops in cells and how this process drives the transformation of a normal cell to a tumour cell as well as other changes in cell morphology. This knowledge will be disseminated through the publication of results in international journal articles as well as being presented at international scientific conferences. As the results obtained have the potential to contribute to an area of research with implications for understanding disease processes in humans, it is likely that results generated will be of interest to a large research community including clinicians and researchers in the pharmaceutical and biotech industries. The work supported by this project is likely to identify additional components and pathways that contribute to the cellular response to DNA Replication Stress and although not directly attributable to this project, it is likely that such insights will prove useful for future projects in the development of new drugs or therapies to treat a range of human diseases.

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

The project will use egg extracts prepared from the unfertilised eggs of the South African amphibian Xenopus laevis. Xenopus can be induced to lay eggs all year round and are therefore able to provide material for experiments as required. The animals are induced to lay eggs by injection of human chorionic gonadotropin (HCG) which is the regulated procedure covered by this project licence. It is estimated that approximately 1500 procedures will be needed to satisfy the requirement for egg extract experiments over the 5 years of the project.

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?

Laboratory bred male and female frogs are kept in a purpose built aquatic habitat in tanks containing 8-10 animals. A water treatment unit circulates water through the tanks. The environment of the habitat is constantly monitored to ensure optimal, temperature and water quality to keep the animals healthy and in good condition. The day before eggs are required, female frogs are induced to lay eggs by injecting HCG under the skin. The animals display little or no distress during this procedure. The instances of adverse effects caused by the injection (infection) are extremely rare. Once egg laying is completed (18-36 hours) the animals are returned to the colony. Male animals provide sperm to be used as a DNA template in DNA synthesis experiments. Sperm production is also stimulated by injection of HCG. 5-8 days after injection, males are euthanized before the testes are removed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

At present it is not possible to reconstitute DNA damage response pathways from purified protein components. Therefore, in order to achieve the project objectives it is necessary to use material obtained from animals. I have investigated and considered the possibilities of using other systems to achieve the objectives of the project. It is not possible to prepare cell extracts from invertebrate systems that possess the range of properties shown by egg extracts made from *Xenopus* eggs. As a result the ability of cell-free extracts from Xenopus to recapitulate cell cycle and DNA damage responses *in vitro* cannot be replicated with the same level of synchrony using eggs from invertebrate species or extracts from mammalian cultured cells. I have also considered other methods of obtaining eggs from *Xenopus* that do not involve regulated procedures; However, the numbers of eggs produced by these techniques are too low to provide sufficient material to achieve the objectives of the work associated with the project.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of regulated procedures (hormone injection) will be kept to a minimum by planning experiments in advance. The volume of egg extract required will be calculated, and hence the minimum number of procedures required can be estimated. Egg production will also be maximized by ensuring that at least three months elapses before an animal is induced to lay again. Typically an animal may be induced to lay eggs 2-3 times a year. In all cases animals are inspected prior to

undergoing the procedure to ensure they are healthy and in good condition. Although freshly made extract is generally preferable for most applications, in some cases previously frozen egg-extract can be used. Therefore the number of procedures required to achieve the objectives of the project will also be further reduced by using frozen extract where possible.

Refinement

Explain the choice of animals 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.

Refinement

Xenopus cell-free extracts are unique in that they will undergo multiple rounds of regulated and highly synchronous cell cycle events in the test tube. While genetic studies provide evidence of the formal relations of components necessary for, or regulating cell cycle progression, the *Xenopus* extract uniquely provides a system in which molecular events can be dissected in the both the context of the cell cycle and development. Moreover, the organisation of DNA damage response pathways in *Xenopus* is comparable to humans, while in lower eukaryotes these are significantly different. A major advantage of the *Xenopus* system over other higher eukaryote models is the capability of rapidly removing protein components and assessing the consequence on DNA replication, DNA repair and cell cycle progression. This strategy has the added advantage of allowing the analysis of proteins essential for cell viability or for development. Many of the DNA Replication Stress Response proteins fall into this category which still represents a considerable obstacle for analysing the function of essential genes in cultured cells. Therefore *Xenopus* represents the only higher eukaryote system where this informative approach is likely to produce satisfactory results with the lowest level of severity of regulated procedure.

PROJECT 32

NON-TECHNICAL SUMMARY (NTS)

Project Title	Preclinical evaluation of CTX DP engraftment in a mouse model of Traumatic Brain Injury
Key Words	Traumatic Brain Injury, Cell transplantation
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Traumatic Brain Injury still remains one of the largest causes of death and disability worldwide. While there have been improvements in management of these patients, we still have no proven treatments available for these patients. There have been numerous trials over the years testing different drugs for patients with severe TBI but none has proven successful. So novel strategies need to be sought to find beneficial treatments for this challenging injury. This project will test whether transplanting stem cells into the injured brain improves outcomes, raising the possibility of using stem cell transplantation as a treatment for TBI. These cells have been extensively researched and used clinically in stroke trials. The question is whether similar effects are seen in TBI, a disease process that shares many of the mechanisms with stroke.

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)?

Ultimately, this work is for the benefit of TBI patients. As there are currently no proven treatments, this work will provide key proof of concept. There are two key questions to be asked. Firstly, how do the stem cells work? Secondly, is there an improvement in mobility. If there is benefit gained in the animals from these sets of experiments, it paves the way towards clinical studies. This would give the necessary information to apply for a clinical trial.

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

Adult C57BL/6 mice are one of the most widely used mice in animal studies due to the consistency between animals and experiments. We will use a maximum of 175 animals over 5 years. The project is planned over 3 years but this includes a contingency in both time and numbers to allow for any unforeseen delay in commencing the project and for any unpredicted events.

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?

While the experimental protocol necessitates a traumatic injury to the rodent brain, the technique used (the controlled cortical contusion injury) is widely used around the world and has been validated. It has gained popularity due to the reproducible injury coupled with the low rates of complications gained from the injury model. The protocol is severe in nature, and possible adverse effects, which would be in exceptional circumstances, include coma and rarely death. The likelihood of this is

rare, and likely to occur immediately after the injury when the animal would still be recovering from anaesthesia. During this stage animals are monitored closely.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

These cell transplantation experiments in TBI require key motor data. One of the important questions is to determine if after injury, there is improvement in motor performance. Subsequently, the only way to assess this is to use animal studies in which an assessment of motor behaviour is assessable. One alternative to animals is to use resected tissue from neurosurgery, to answer if human cells respond to the stem cells. We will also do this, but this does not give us the necessary motor information.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will reduce the number of animals by designing the experimental as a two stage process. The most important experiments are performed first. Should the information be equivocal, then further studies will be performed. Should the information be either clearly negative then no further experiments will be performed. Animals will be used for several tests and also for brain tissue analysis, thus minimising the numbers needed. We have used 'power analysis' to calculate the number of animals for our experiments to ensure the maximum chance of a positive result with minimal numbers of animals.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the most established mammals in animal research due to their consistency and reproducibility. They are consistent when it comes to behavioural analysis. The injury model has been validated in rodents by numerous groups around the world. Animal welfare is ensured by appropriate anaesthesia, analgesia and importantly close monitoring by trained and dedicated staff to ensure the absolute minimal harm to these animals. In drafting this protocol, the ARRIVE checklist has been used as a guide to ensure the maximum welfare of the animals and adherence to national and international guidelines.

PROJECT 33

NON-TECHNICAL SUMMARY (NTS)

Project Title	Circadian regulation of processes underlying chronic inflammation
Key Words	Biological clocks, Inflammation, Arthritis, Immune system, Colitis
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

The biological clock is an internal timing mechanism which allows animals and plants to live and thrive in the 24 hour environment generated by the Earth's rotation. Individual cells possess clockwork machinery which allows them to "tick" in a 24 hour fashion. This project focuses on the role of clocks within immune cells - specialised cells which help to fight infection and keep animals healthy. Work outlined here will investigate how the clocks within these cells regulate the inflammation which is associated with chronic inflammatory disorders such as rheumatoid arthritis and chronic inflammatory bowel disease. These types of diseases affect approximately 1-2% of the UK population and can have devastating effects on quality of life. Evidence from patients suggests that these chronic inflammatory diseases may be regulated by the biological clock - for example rheumatoid arthritis patients often report increased joint stiffness in the morning.

The work outlined in this project will investigate how the biological clock regulates the processes which cause chronic inflammation. This will generate insight into how chronic inflammatory diseases might be better treated in the clinic. Although there are several different types of drugs used to treat chronic inflammatory diseases, these are not always effective in every patient, and many are associated with negative side effects. This work is aimed at finding new targets for which to develop drug treatments, and also to establish whether by taking existing anti-inflammatory drugs at certain times of the day we can improve how effective they are.

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

This project will bring about a greater understanding of how the body's biological clock regulates the disease processes which cause chronic inflammatory diseases. It is hoped that in the long-term this will advance the way in which these disorders are medically treated. We hope to identify new targets for drug treatments, but also consider the possibility of altering how current therapies are utilised. For example, by administering a drug at a specific time of the day we may be able to improve how effective it is in treating the disease, and also reduce the occurrence of unwanted side-effects.

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

This project will utilise approximately 9050 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 levels of severity? What will happen to the animals at the end?

Breeding: This project covers the breeding of a number of genetically altered lines of mice which have alterations in their biological clock. This includes animals in which a key part of the clockwork machinery has been deleted from either the whole animal or just one particular cell type. These lines of mice are unlikely to show any adverse effects. Additionally, mice will be bred which spontaneously develop arthritis as they grow older. This is likely to cause a moderate degree of discomfort and pain. These animals may be used to look at how the disease changes at different times of day, or to harvest blood which can be purified and administered to other animals as a way of inducing arthritis. Induction of arthritis: This project will use several different ways of inducing arthritis, each of moderate severity. Either by injecting biological components derived from other animals or injecting collagen or an antigen under the skin. Each method results in the animal developing localised inflammation and swelling within the paws or knee. Samples will be taken from these animals either during the disease state (e.g. blood samples) or at the end of the experiment (e.g. cells and tissue). This arthritic state will cause a moderate degree of discomfort and pain. Animal developing severe inflammation will be removed from the study and humanely killed. Induction of chronic inflammatory bowel disease: Mice may be administered a chemical in the drinking water which causes inflammation within the gut, resulting in a moderate severity disease much like chronic inflammatory bowel disease in humans. This results in weight loss and diarrhoea. Treatment periods with this chemical will be limited, and the experiment will be ended if animals show signs of severe weight loss. Surgical intervention: Animals may be surgically treated in a number of instances. Firstly, devices may be implanted under the skin which record the animal's body temperature and activity. Secondly, a small pellet may be implanted under the skin which slowly releases a hormone. Thirdly, a small incision may be made in order to administer a brief pulse of light to organs of the immune system with the purpose of changing the colour of immune cell populations to enable cell tracking through the body. Finally, the adrenal glands may be removed in order to abolish the rhythmic release of the anti-inflammatory hormone corticosterone into the blood. Corticosterone may be replaced through timed administration to either invert these rhythms, or maintain levels at the nadir or peak. All of these procedures are well tolerated and not associated with any adverse effects. Through the use of proper surgical techniques and administration of antibiotics, post-operative infections will be avoided. Pain relief will be provided during and after surgery. At the end of the experimental protocol, animals will be culled using an appropriate and human method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The animal studies outlined in this project will be supported by additional techniques which utilise cells harvested from either naive, healthy mice, including animals genetically altered to affect their circadian rhythm, or from humans.

Animal models are essential to address the research questions raised in this project. Computational modelling and *in vitro* techniques are insufficient to model the complex interactions between the mammalian circadian clock and the immune system.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Animal husbandry

Where our studies involve breeding lines of mice which have been genetically altered, we take care to breed the minimum number of mice possible to provide us with animals to use experimentally, but also to maintain an efficient breeding colony. This is achieved by effective communiction and co-ordination with our staff in the animal unit, and by keeping up-to-date accurate records.

Laboratory techniques

By taking advantage of the large array of cutting edge technologies available to us, we aim to obtain the maximum information possible from the fewest animals possible. Laboratory techniques which we routinely use allow us to generate large data sets from single samples and thereby reduce the need to repeat experiments to generate more experimental tissue.

Experimental design

Experimental design is critical to reducing the numbers of animals used. A statistical expert has been consulted regarding the planning of this project and has helped in informing the correct statistical tests to use in order to generate robust reliable conclusions.

Refinement

Explain the choice of animals 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.

Refinement

Choice of animal models

1. Inflammatory arthritis.

Mouse models of inflammatory arthritis are widely studied models of the human disorder rheumatoid arthritis. These models produce a disease characteristically very similar to the human condition. Such models are often used in the early stages (pre-clinical) of the development of new drugs to treat rheumatoid arthritis. These models are classified as moderate severity, and provoke inflammation in the joints, which will lead to a degree of pain and discomfort in the animals. Due to the nature of the study, it will often not be possible to use analgesia during this phase as such drugs themselves affect inflammatory responses and would severely compromise the disease model. Once mice begin to show signs of arthritis, they will be maintained in this phase for the minimum time possible in order to achieve the objective outlined in this project.

In order to minimise suffering, animals showing excessive signs of joint inflammation (as determined by regular assessment using a severity scale) will be removed from the experiment and be humanely euthanised. Mice will be group housed and provided with soft nesting material and environmental enrichement wherever possible.

2. DSS induced colitis

DSS induced colitis provokes an inflammatory response within the gut. We aim to optimise the dosing schedule to induce a mild/moderate localised chronic inflammation, which is essential to address our research goals. Animals will be monitored regularly for signs of ill health (substantial weight loss, loss of condition) and will be held in this chronic inflammatory state for the minimum time possible in order to achieve our research objectives.

3. Surgical procedures

Where it is necessary to undertake a surgical procedure, this surgery will be performed under aseptic conditions. Pain relief will be provied to these animals, and animals will be group housed and provided with environmental enrichment.

General welfare measures

Animals will be group housed wherever possible, and provided with environmental enrichment. When an animal is undergoing a procedure, they will be monitored regularly for disease progression (where appropriate) and for signs of adverse effects. Animals undergoing surgical intervention will be provided with p ain killers, antibiotics and extra food if necessary.

PROJECT 34

NON-TECHNICAL SUMMARY (NTS)

Project Title	Breeding and maintenance of GA and HM animals
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;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The primary purpose of the work conducted under this licence is to enable the breeding of rats and mice with genetically altered (GA) or harmful mutations (HM) for use by scientist in non-regulated procedures, (e.g. for tissue harvesting following schedule 1 killing). In addition this licence may be used to facilitate the introduction of new GA or HM lines, for use in other project licences, using frozen embryos or sperm or to use these techniques to improve the health status of established breeding lines.

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 animals bred under this licence will be used to provide tissues for use in medical and fundamental research.

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

We anticipate breeding 2000 mice and 200 rats per 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?

No adverse effects are expected in the vast majority of the animals bred. Most of the genetic alterations or mutations are not expected to result in discernable suffering. In a small number of cases it may be necessary to bred animals with genetic alterations that have the potential to compromise the wellbeing of the animals. In such cases animals will either be culled at an age below that at which adverse effects occur or as soon as any sign of the adverse effect is detected. The vast majority of the animals will be bred using conventional methods and killed in order to obtain tissue for use in medical research.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

This is a breeding licence for GA and HM animals, the vast majority of which will be used in non-regulated procedures. There is no alternative to the use of animals for this purpose.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The studies in which these animals are used will have undergone a full ethical review requiring a statistical justification of animal numbers. Wherever possible animals will be reared as homozygous lines to minimise the numbers required.

Refinement

Explain the choice of animals 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.

Refinement

The majority of animal used will be mice, which is the least sentinel mammalian species suitable for the purpose. Rats will only be used where suitable mouse models are not available. The genetic alteration are not expected to result in any suffering. The animals will be reared in high health status facilities, provided with environmental enrichment and group housed wherever possible.

The animals will be bred in accordance with the Home Office GAA Assessment Tool. The tool makes recommendations which improve the welfare of the genetic mouse and contributes to the efficient running of a breeding establishment.

PROJET 35

NON-TECHNICAL SUMMARY (NTS)

Project Title	Understanding host-pathogen interaction in a zebrafish model
Key Words	Infection, bacteria, fungi, zebrafish, antimicrobial resistance
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

Infectious disease is still the world's biggest health problem. Antibiotic resistance is a growing problem and identifying new antibiotics has been very difficult. This project aims to understand how our immune system is able to fight infection normally, what goes wrong when it cannot and how we might be able to aid the immune system in fighting infection.

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

This project will advance our knowledge of the immune system and how infections happen in people whose immune systems to not work correctly. Using this knowledge, we will try and find new ways of treating infections by helping the immune system. Helping the immune system is a treatment could work with, or replace, existing antibiotics reducing the need for new antibiotics.

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

We expect to use 21,225 zebrafish over 5 years.

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

Almost all of these animals (20,000) will be used for breeding only (mild severity) and expect there to little or no adverse effects from breeding. These animals will be kept until they reach the end of their healthy life span (usually between 2 and 3 years). Once these animals show signs of significant aging they will be culled using an overdose of aesthetic. A much smaller number (1,100) will be used to study infection in protected animals. Our experiments are designed to understand how infections are removed by the immune system and that most animal will only experience mild severity. Any infected animals that show clinical signs beyond mild severity will be culled using an overdose of anaesthetic.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

How the immune system responds to infection and how this changes the outcome of infection cannot be understood without the use of animals. It is not possible to recreate the complexity on animal infection (with all the relevant cell types and tissue environments) with alternative approaches currently. Almost all of the experiments we will do in this project will be in non-protected animals (zebrafish larvae). Protected animals will be used to breed fish with genetic modifications.

Where we are using protected animals for infections this because we are studying parts of the immune system that are only developed after the larval stages of zebrafish are not present at all in non-protected alternatives.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have used our experience of non-protected alternatives to ensure that we are using the minimum number of animals. Almost all animals will be used for breeding only over their healthy lifespan. For our pilot studies of infections in protected animals we have used our published data in non-protected animals to predict group sizes needed to generate statistically significant data as evidence for extending our infection experiments into protected animals.

Refinement

Explain the choice of animals 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.

Refinement

Zebrafish have a similar immune system to our own but have much lower neurophysiological sensitivity in comparison with other animal models and is therefore the most refined model for this project. Almost all of our experiments will occur in non-protected animals. In our infections of protected animals, we are minimising harm by culling animals that show clinical signs of infection. Other procedures are very unlikely to result in harm but we will closely monitor all animals and will end procedures, treat or cull by an overdose of anaesthetic if needed.

PROJECT 36

NON-TECHNICAL SUMMARY (NTS)

Project Title	Molecular mechanisms of neural development
Key Words	Embryo development, Boundary formation, Nervous system
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The complex organisation of the nervous system forms progressively during embryo development. The project focuses on two general aspects of nervous system development that are only partly understood:

(i) How sharp borders form between different regions of the nervous system. During development, many tissues become subdivided into regions that each form a different set of cell types. To generate a precise tissue organisation, it is essential that sharp borders form between these regions. The project focusses on a family of proteins that mediate cell signalling required to form many borders.

(ii) How the transition of stem cells to become nerve cells is controlled in time and space in the embryo. This transition involves an interplay between activating and inhibitory factors, including cell-to-cell signals and proteins (termed transcription factors) that switch genes on or off. The project seeks to understand how specific inhibitory factors function. A major goal is to identify the genes that they control.

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 benefits of this Programme are to advance knowledge of fundamental mechanisms of nervous system development, and have long term medical implications. The mechanisms studied are involved in the normal homeostasis of tissues in animals, including humans, such as in the regulation of stem cell differentiation and maintenance of tissue organisation. When these mechanisms are disrupted they lead to diseases such as the metastatic spreading of tumour cells.

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

The Programme will use approx. 75,000 adult fish over 5 years.

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

The transgenic and mutant animals are not expected to exhibit a harmful phenotype. The level of severity is therefore mild. However, it is not possible to fully predict the nature or severity of any potential defect. If a recessive mutant has a harmful phenotype in homozygous adults, they will be maintained as heterozygotes. Dominant harmful mutations are not required in the project, and should any arise, the animal will be killed by a Schedule 1 method. All of the Regulated Procedures are in one Protocol for the generation and breeding of mutant or transgenic zebrafish to

generate embryos; a minority of adult fish are subjected to fin clip biopsy for genotyping.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The experiments require use of animals since developmental mechanisms involve complex networks of gene control and cell signaling that are only partly understood and cannot be reproduced using in vitro systems. For studies of mechanisms of cell segregation and border formation, the project uses cell culture and computer simulations.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used is minimised by application of incisive techniques that include transient knockdown of specific genes, generation and use of mutant lines, use of sophisticated transgenic methods to achieve temporal or spatial regulation of expression, and use of fluorescent reporter expression in the eye to reduce need for genotyping of transgenic lines. The number of animals used is determined by the need to achieve statistical significance in the results. The majority of experiments involve transient alteration of gene function in wild type embryos and are not Regulated Procedures.

Refinement

Explain the choice of animals 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.

Refinement

Zebrafish is the organism chosen for the project as it is amenable for targeted gene manipulation and generation of transgenic reporter lines, and the transparency of the embryos enables live imaging of cells. Specific questions are addressed by assays using established cell lines in cell culture in combination with computer simulations; the results of these studies enable refinement of the experiments in zebrafish.

PROJECT 37

NON-TECHNICAL SUMMARY (NTS)

Project Title	Influenza in small animal models
Key Words	Influenza, Antigenicity, Ferret, Transmission, Vaccines
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The first aim is to generate panels of post-infection or hyperimmune ferret antisera with which to examine the antigenic characteristics of circulating influenza viruses of humans and animals and to analyse the antigenic characteristics of viruses for use in influenza vaccines. Other aims are to use ferrets to examine the transmission of animal or zoonotic influenza viruses between ferrets, and to examine the efficacy of new influenza vaccines and antiviral medicines.

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)?

Influenza vaccines are made to be most similar antigenically to the viruses in circulation. The use of small animals in which to generate the antisera and ensuring a close antigenic relationship between the vaccine and the circulating virus are critical to this and ferrets are the best small animal to use. Ferrets are also the best small animal for the study of the sensitivity of virus to experimental vaccines, new medicines and estimation of the threat from animal influenza viruses.

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

Up to 450 adult ferrets are proposed to be used for these studies over five 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?

It is possible that some of the experimentally infected ferrets will develop severe illness, for example with zoonotic influenza viruses such as H5N1 and H7N9 avian influenza viruses. Seasonal human influenza infection of ferrets is generally not severe. All experimentally infected animals will be monitored during the experiments and thy will be treated with antiviral medicines when infection is deemed to be severe.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The response of the ferret to infection with influenza viruses is remarkably similar to that observed in humans: disease signs are similar, transmission is similar and the immune response to infection is similar to that seen on infection of naïve human

subjects. Therefore the ferret is considered the best small animal model of human influenza.

Alternatives to ferret antisera for assessing virus antigenicity are continually considered, e.g. mouse or human monoclonal antibodies. Mouse monoclonal antibodies are inferior to the polyclonal antibody response of a ferret to infection with influenza viruses for the characterisation of the antigenic properties of emerging influenza virus isolates. The suitability of human monoclonal antibodies to complement post-infection ferret antisera is a topic of our recent and current research and future research plans.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of ferrets to be used is not large. The number reflects the number of new sera (or replacement sera) used each year raised against reference influenza viruses. Over the past two years post-infection ferret sera against on average 55 new reference viruses have been produced each year.

Experiments to monitor transmission between ferrets also use small numbers of animals. The results of evidence of transmission between ferrets, particularly by respiratory droplets, is a key parameter of the risk assessment that a novel virus might have human pandemic potential.

Experiments to carry out investigation of the likely effect of vaccination or the efficacy of antiviral medicines in vivo, complement in vitro assays. It is anticipated that these studies will be carried out only when a novel influenza virus emerges that is considered to be a particular risk to humans either as a severe zoonotic infection or as a potentially human pandemic virus.

Refinement

Explain the choice of animals 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.

Refinement

The ferret has been the small animal model of choice for influenza virus work since the virus was first isolated in ferrets in 1933. The dynamics of the ferret infection and the ferret's response to infection are very similar to those seen in humans. Other alternative species that may be suitable include monkeys and, for some experiments, guinea pigs but the ferret has a long history of very satisfactory use with influenza viruses that infect humans. Following infection ferrets will be

monitored daily and antiviral medicines will be used should severe disease signs be observed.

PROJECT 38

NON-TECHNICAL SUMMARY (NTS)

Project Title	Evaluation of Biotherapeutics for Paediatric Cancers
Key Words	Adoptive transfer, Vaccination, Cancer, Paediatrics
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

This project is designed to improve understanding of how immune based therapies can be employed to effect responses in human solid cancers. Although cure rates in childhood cancers have improved dramatically over the last 20 years with the introduction of multimodality therapies incorporating systemic chemotherapies, there remain certain recalcitrant solid tumours with very poor prognosis. Our research is primarily focussed on high risk childhood brain and nervous system cancers as well as high risk leukaemias. In high-risk neuroblastoma, patients have widespread metastatic disease but with 1 year of intensive and painful multimodality treatments, long term cure remains less than 50%. More recently, significant improvements in disease control have been achieved through the introduction of immunotherapy (monoclonal antibodies). In high risk childhood brain tumours essentially no progress has been made in treatment in the last 40 years. Current research priorities are to identify more targeted therapies using antibodies and genetically modified cells of the immune system

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)?

Through this work, clinical trials of experimental immunotherapy in children with cancer are designed and refined prior to the treatments being given to patients. Immunotherapy works by manipulating the relationship between the tumour and the host organism’s response against it. It is essential therefore to understand this relationship and how it can be manipulated using experimental systems with normal immune systems. Therefore our research largely uses mice with normal intact immune systems because the great similarity between mouse and human immunology. Where possible experimental proofs of principle are performed using human cells and cell lines before exploring whether similar experimental manipulations have the desired effects in the animal model. To achieve these aims mice will with small tumours will receive treatments for the tumour through injection of drugs or cells. Mice may suffer toxicity from the treatments or the tumours themselves but experiments are terminated if tumour growth exceeds nationally agreed limits or if there is evidence of animal ill health.

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

Approximately 2,600 mice over 5 year period. This number includes approximately 400 mice derived from breeding which do not have harmful mutations (by chance) and are unlikely to be used in any of the listed protocols. Mice are selected as they are the organism most suited to evaluation of immunotherapy for cancer; many

similarities between mouse and human immune systems and similar patterns of tumour growth and responsiveness.

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?

Mice are kept in cages of between 5 and 10 animals provided with bedding, feed and water by bottle. Some mice will develop spontaneous tumours and used in evaluation of tumour biology as well as efficacy of immunotherapy interventions Animals will be dosed by injection of tumour cells or therapeutics agents by injection of small volumes of liquid (normally maximum of 0.3 of millilitre). These injections can be under skin into muscle and into the abdominal cavity. In studies of some brain tumours there may be injection into the brain through an existing small hole in the skull, or into the kidney. For kidney injections mice will be anaesthetised under general anaesthesia and a small incision made in the flank to gain access to the kidney. A small volume of tumour cell solution is injected under the capsule of the kidney. The skin will be sutured after the operation which lasts 10 to 20 minutes. For injection of tumour cells into the brain, mice undergo general anaesthetic and a rigid frame holds both mice and syringe in place to allow consistent administratin of accurate volume of tumour cells into identical positions. Mice will develop tumours that reflect the human (childhood) counterparts and will be administered agents to reduce tumour growth. The procedures used in each tumour-bearing animal include the administration of drugs or cells to induce tumour shrinkage. These are usually administered by injection under the skin or into the abdominal cavity using a small needle and small volume of fluid. . Mice are held in position for these procedures, which are given without anaesthetic and take only a few seconds to administer. Mice will also undergo periodic bleeding to assess response to therapies or to evaluate tumour growth. For this the mouse is usually not anaesthetised although where possible blood tests will be taken during a general anaesthetic administered for other reasons (for example for tumour imaging). The bleeding procedure invoves placement of the mouse in a restraining tube and placement of a needle in a vein at the base of the tail. The procedure takes only one or two minutes. Similarly this tail vein injection can be performed using identical technique to administer tumour cells or anti cancer drugs or cells. Some mice will also undergo imaging of tumours. This involves a general anaesthetic to keep the very still whilst being place in an imaging machine. At the end of the experiment or if there is uncontrolled tumour growth or side effects of treatment, mice will be killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Non animal alternative are used for initial confirmation of likely efficacy of an intervention/therapeutic to be evaluated. However the capacity of the intervention to overcome the barriers presented by the intact tumour system is the major impediment to the development of effective immunotherapies. Currently animal models are the best models of the tumour microenvironment that are likely to be reflective of the human counterpart

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

1) Experiments are designed so that initial dose finding experiments give an initial indication of potential of a therapeutic intervention to show efficacy. These preliminary experiments allow predictive calculations to be made that will show the minimum number of animals per treatment group are likely to give a highly significant result that does not need to be repeated.

2) Where possible non-invasive imaging of tumours and infiltration of transferred cells into the tumours is used; this makes it possible to make assessments at numerous time points in a single animal thereby greatly decreasing the total numbers of animals needed for a full data set to be obtained

Refinement

Explain the choice of animals 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.

Refinement

Mice are the only species to be used. They have an immune system that is very well characterised and highly homologous to the human counterpart, which makes them an ideal species for evaluation of biotherapeutics.

Disease models are of four main types; 1) mouse tumour cell lines implanted in mice with intact immune systems , 2) transgenic (genetically modified) mice with spontaneously occurring tumours in the context of an intact immune system, 3) human cell lines injected subcutaneously or intravenously in immunodeficient mice and 4) human primary neuroblastoma cancer stem cells injected into the kidney capsule which reflects the normal site of tumour growth in the human situation. Each model has advantages and disadvantages in terms of similarity with the human disease and therapy counterpart. The use of human primary cancer stem cells has particular advantage that the activity of the patient’s own cells against their own

tumour can be evaluated. In each experiment the approach that is best suited to provide relevant information for human disease with least animal harm will be selected.

PROJECT 39

NON-TECHNICAL SUMMARY (NTS)

Project Title	Phenotyping of glucose metabolism
Key Words	Metabolism; diabetes; glucose homeostasis; obesity.
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Diabetes, obesity and cardiovascular disease have become major health problems with very significant costs to the individual and society. To improve the health of individuals and reduce the burden of diseases linked to diabetes and obesity we need to achieve a greater understanding of the mechanisms that underlie these conditions. It is becoming increasingly clear that the mechanisms involved in regulating glucose and energy metabolism also play much wider roles in cells and, for example, are linked with immune and stress response systems as well as with the development and treatment of diseases such as cancer. As a result, research groups working in apparently distant fields of science are increasingly finding effects of their candidate gene or protein of interest on whole body glucose or energy metabolism. This then leads them to think as to why this might be important and whether new treatments could emerge from a better understanding of these effects. However, these science groups are often highly specialised in one area and not skilled in the study of glucose and engery metabolism. In addition, even groups that study diabetes and obesity are rarely trained in the highly technical procedures that are required to conduct detailed characterisation (phenotyping) of rodent models.

The purpose of the [REDACTED]is to provide an in vivo laboratory with state-of-the-art equipment and highly skilled technical staff to perform studies in collaboration with other investigators. This would also ensure that the most refined experimental procedures were carried out, using the minimum number of animals and producing scientific data that are robust, highly informative, and allow for comparison between different research groups. A step-wise approach will be adopted in order to ensure that interventions are only as invasive as can be justified. In the first step, collaborators will be expected to have authority on their own project licences to provide prelimary evidence that metabolic phenotyping is warranted, (e.g. raised fasting glucose level or unexpected obesity). Only when these first line tests are significantly different from “normal” will procedures under this licence be invoked. Metabolic phenotyping involves a number of potential procedures ranging from simple assessment of blood glucose or body weight to detailed glucose clamp studies which require insertion of long-term catheters into blood vessels under general anaesthesia. All are conducted under sterile conditions and use standardised protocols, with full attention to pain relief and avoidance of stress. The ultimate aim is to provide detailed information to investigators about the role played by their candidate gene/protein in glucose and/or energy metabolism which may lead to the future development of new therapies.

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

This project aims to help investigators study new rodent models in the context of human diabetes and or obesity and that will lead to an improved understanding of these complex diseases. Through provision of a focus of technical and scientific expertise, investigators are able to access individuals who can help with study design relevant to the questions they ask. This along, with the ability to perform highly complex procedures reduces the likelihood of inappropriate or unnecessary studies thus reducing the number of animals required for a given investigation. In addition, expertise in performing the metabolic studies ensures that fewer animals are required to adequately study a given parameter (e.g. glucose clamp study). The information taken from these studies offers the chance of developing new therapies derived from the work of investgators in many centres focused who focus on diabetes and metabolism but also those focused on other areas such as inflammation and cancer.

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

Rats and genetically altered mice will be studied in this proposal. Approximately 4000 mice and 1000 rats will be bred, of which 2000 (mice) and 750 (rats) might undergo further regulated procedures, over 5 years.

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

The genetic alterations themselves are not expected to cause adverse effects but may influence whether the mice become diabetic (e.g., after being fed a high-fat diet). Regular observation and recording of clinical condition, blood glucose and body weight, ensures that all animals are monitored closely. A number of procedures are described in the license for which expected adverse effects, refinement controls and humane end-points have been clearly described and are intended to ensure that animals are monitored closely. All procedures are of the mild or moderate category. On completion of metabolic phenotyping studies animal are killed humanely and tissue may be taken for biochemical analysis.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Collaborators will be expected to have established robust hypotheses (from in vitro studies or their own work with animals) about the role of glucose/energy metabolism

or cardiovascular function in the systems they study. Because of the complex interactions both between and within several organs (e.g. fat, muscle, liver, pancreas. CNS) it is impossible to fully model whole body glucose/ energy metabolism, cardiovascular and endothelial function completely in vitro. Rodents have been chosen for our studies because this species has been extensively used as a model organism in the understanding of human metabolic diseases. Importantly, gene targeting technology and dietary manipulation is widely available for the mouse and thus allows investigators to precisely establish the relationships between genes and biological processes.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The decision path will involve animal numbers determined by appropriate power calculations at each experimental stage. Only if these showed significant deviation from expected values would other studies be carried out. All studies will be performed by experienced members of staff following standardised protocols. This will ensure minimal variability and maximal consistency and reliability, thereby reducing the number of animals required to achieve meaningful data.

Refinement

Explain the choice of animals 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.

Refinement

The decision path involves an escalation of intervention, but the more invasive steps will only be followed when there is good evidence that the scientific value is high.

The insertion of chronic vascular catheters will be performed under general anaesthesia by highly skilled surgeons who have several years of experience in this complex technique. Careful post-operative monitoring and pre-emptive and post-operative analgesia will be in place to ensure minimal suffering. The catheters allow for painless blood sampling in an awake, unstressed and unrestrained animal; crucially important if the clamp experiments are to be robust and meaningful. The data gained cannot be obtained using terminally anaesthetised models.

Metabolic phenotyping means that multiple procedures may be performed in any single animal. This greatly reduces the number of animals required. In the unlikely event that an animal shows distress during the phenotyping protocol, it will be removed from the study and euthanized. The event will be discussed with the NVS in

order to identify whether modifications are required to minimise reoccurrence of the issues identified.

All testing will be performed by experienced members of staff, following standardised protocols thereby minimising variability and ensuring that the data produced is of a high quality and reproducible.

PROJECT 40

NON-TECHNICAL SUMMARY (NTS)

Project Title	Improving Welfare of Farmed Pigs
Key Words	Pigs, Welfare, Behaviour, Stress, Physiology
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Commercial pig production can result in a variety of challenges to pig welfare. We use behavioural and physiological indicators of welfare to investigate the causes and consequences of these challenges.

Specifically we seek to 1) Understand how early life environments affect immediate and longer term welfare and other outcomes for piglets. 2) Improve prevention and prediction of tail biting, and 3) Understand how social stress impacts on welfare and influences gut health through changes to populations of micro-organisms, such as bacteria, living in the gut.

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

Our research leads to: i) guidance on best practice for pig breeders, pig producers and veterinarians, or ii) new technologies or techniques to be used on-farm iii) evidence base for policy makers and assurance schemes devising rules and recommendations for keeping pigs. Farmed pigs benefit through improved welfare, and this is seen as a benefits by citizens and consumers; pig producers may benefit through more efficient production with fewer loses (e.g. piglet mortality).

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

Commercial breeds of domestic pig will be used, varying in age from neonates to mature adults (depending on the study). An individual study may involve as few as 20-30 pigs or as many as 1200 (expected total over 5 years no more than 1780).

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?

In early-life studies pigs will undergo physiological and behavioural data collection where some of the protocols may cause some acute periods of pain (e.g. tagging for tissue collection) or distress (e.g. restraint for tissue collection, negative stimulus in conjunction with behavioural testing). Procedures will only be carried out by trained or supervised staff and where necessary appropriate pain relief will be administered. Clear end-points are in place for behavioural tests that involve periods of solitude, restraint or the application of a negative stimulus (e.g. restraint). In tail biting studies, tail biting outbreaks are expected, and lead to tail injury and infection risk. Pigs will be closely monitored and when an outbreak occurs, affected pigs will be immediately removed for appropriate treatment (e.g. analgesia, antibiotics) to enriched housing, and monitored to ensure that no further tail biting occurs. Pigs which have experienced no lasting adverse effects, or have recovered from earlier adverse

effects (e.g. healed tail injuries) will remain at the establishment until they reach slaughter age. Pigs with total tail loss or signs of secondary infections will be humanely euthanised. For gut health studies, pigs will be humanely euthanized for collection of tissues. In social stress studies, pigs reared under commercial stocking density, and mixed with unfamiliar animals may experience stress and injury as a result of aggression. Any severely bullied pigs will be removed from the pen and any injured pigs will be appropriately treated or euthanized on the advice of the NVS. During isolation for behavioural testing, some individuals may become stressed and continually try to escape- these pigs will have their test ended and returned to their home pen.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We aim to inform on the welfare of pigs kept in commercial situations and no alternatives exist. The welfare problems we study require the whole living animal to be studied (e.g. to record behavioural responses), and non-animal alternatives are not available. Making use of the literature on behavioural and physiological responses in pigs and other mammals (as model species) will ensure that we build on existing knowledge and develop focussed hypotheses before beginning animal studies in the pig. We also carry out various studies which are positive or neutral for the pigs, so are not regulated. These studies are part of our larger body of work which informs the regulated studies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Animal use will be reduced by using efficient experimental designs incorporating factorial design, blocking and randomisation, and by minimising animal numbers by using power analysis based on previous similar studies where information is available. Our studies are reveiwed by an independant statistician.

Refinement

Explain the choice of animals 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.

Refinement

Studies of pig behaviour and welfare require the use of pigs. We use a number of refined methods developed over the years. For example, where possible: pigs are habituated to handling prior to behavioural testing, saliva sampling rather than blood is used, and ear tissue collected for DNA as part of routine ear tagging for ID purposes. Pigs are closely monitored when negative outcomes are expected (e.g. in studies of tail biting) so that they can be treated as soon as possible. Analgesia will be used to control pain in all studies where this is expected.

PROJECT 41

NON-TECHNICAL SUMMARY (NTS)

Project Title	Viral and non-viral gene therapy
Key Words	Cystic Fibrosis, Haemophilia, Pulmonary alveolar proteinosis, Gene Therapy, Cell Therapy, Thrombotic thrombocytopenic purpura
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

We are developing novel treatments for a range of diseases that currently have insufficient treatment options and are affected by high treatment burden and/or pre-mature mortality. These include cystic fibrosis (CF), alveolar proteinosis, thrombotic thrombocytopenic purpura and haemophilia.

Over the last 20 years [REDACTED]which showed that gene therapy can stabilise CF lung disease. However, we have to further improve the efficiency before gene therapy can be licenced as CF drug.

[REDACTED]gene transfer to the lungs and nose of mice can lead to protein release from the lungs and nose into the blood. This data opens the possibility of using the lungs as factories for proteins required in the blood.

For example we envisage to produce FVIII, a protein that is lacking in haemophilia patients, in the lung.

Importantly treatments for haemophilia are currently suboptimal and many patients don’t have access to regular treatments.

Furthermore, we aim to produce ADAMTS13, lacking or deficient in TTP individuals, in the lung of acquired TTP mice or ADAMTS13 knockout mice. Currently TTP individuals have a high treatment burden and rely completely on donor plasma which is associated with high morbidity.

[REDACTED]lung gene transfer leads to production of secreted protein in the lung. This data opens the possibility to apply our gene transfer vectors to a wide range of other lung disease including pulmonary alveolar proteinosis (PAP). PAP patients suffer from lipid accumulation in the lung and currently no good treatment options exist.

In addition to applying the gene transfer agents directly to the lung we will also assess if cells that have been gene corrected outside the body can be reimplanted into the lung and lead to efficient production of therapeutically relevant proteins.

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 development of new therapies may significantly improve survival and quality of life in patients with cystic fibrosis, haemophilia, TTP and pulmonary alveolar proteinosis

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

Mice and rats. We predict to use approximately 2500 mice or rats per year, but actual numbers will vary depending on the phase and the success of the individual projects.

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

The vast majority of our procedures are mild (>90%) and will not cause severe adverse effects in the animal. The cystic fibrosis knockout mouse can suffer from intestinal problems, although we will use “gut-corrected” CF mice that do not suffer from intestinal disease. Some mice may be treated with pathogens know to cause lung diseases (eg Paseudomonas aerugiosa which is a common pathogen in cystic fibrosis patients). Some of our infection models may suffer from lung inflammation which may lead to adverse events such as weight loss and reduced mobility which will be carefully monitored and minimised wherever possible by using the lowest suitable bacteria dose. To assess the efficiency of new treatments for haemophilia it is important to quantify whether blood clotting time is reduced after gene therapy. The assay can only be done in living mice, because it requires complex interaction of many proteins involved in blood clotting which cannot be mimicked in other models. Haemophilia mice suffer from prolonged bleeding time. Adverse effects may include prolonged blood loss. This will be mitigated by quantifying blood loss and terminating the experiment if blood loss reaches a defined threshold. The mouse model for pulmonary alveolar proteinosis (PAP) has near normal life expectancy, but shows lipid accumulation in lungs, but not in other organs. We do not expect any significant side effects in this model. Treatment of PAP with a gene transfer vector may exacerbate the symptoms, but as described above this will be carefully monitored and acted upon appropriately. Procedures will be carried out using non-recover anaesthesia or allow animals to recover from anaesthesia as appropriate. We have no direct experience yet using a tail tip assay to monitor blood clotting time in haemophilia mice, but have and will further seek advice from collaborators. Based on available information we expect that an effective blood clot will form and bleeding will recede within 10 min. However, until we have gained more experience we will perform these procedures under general anaesthesia without recovery. At the end of the experiments animals will be humanely killed.To assess the efficacy of gene therapy for TTP, a living animal must be used to understand the complex interplay of proteins and cell types contributing to haemostasis and thrombus formation. ADAMTS13 knockout mice and acquired TTP mice are healthy and have normal clinical symptoms, except for when challenged with ultra-large VWF which induces TTP-like symptoms in the mice. These mice display changes in behaviour and a range of haematological symptoms which can be detected through blood sampling, histological analysis and observations. Successful treatment will prevent onset of

these symptoms and restore ADAMTS13 expression. According to literature, mice will revert to normal behaviour and clinical symptoms within 3-14 days following TTP induction. Following TTP induction, mice may exhibit severe symptoms, despite not being reported previously, thus animals will be monitored twice daily for 14 days or until mice return to normal behaviour and humanely euthanized in the event of severe symptom presentation.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Over the years we have learnt that the efficacy of gene transfer to the lungs can only be assessed in an intact organism because extra- and intracellular barriers and importantly inflammatory and immune responses cannot be reliably mimicked in cell culture systems despite our extensive attempts. However, we have access to several human lung ex vivo models including freshly extracted airway cells and cells grown at an air liquid interface which we utilise in parallel to working with mice. We are also aware of a more recent
model, which involves growing airway cells and blood vessel cells on smallchips which we may be able to utilise when more widely available.

In ADAMTS13 knockout mice, there are no clinical symptoms unless the disease is induced through administration of ultra-large VWF protein. This stimulus is required to trigger TTP pathogenesis which closely recapitulates how TTP individuals need a second vascular insult to trigger an acute TTP episode. These acute clinical symptoms cannot be reproduced in an *in vitro* model, however prior to using mice, gene transfer will be established in *in vitro* and *ex vivo* ALI models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We use inbred strains wherever possible as inbred animals are effectively identical which reduces experimental variability and minimises numbers of animals used. We seek statistical advice to ensure experiments are appropriately designed to achieve research objectives with the minimum number of animals. We will breed “on demand” to avoid over-breeding. To reduce bias and confounding factors animals will be randomly assigned to
treatment and control groups at the start of an experiment. In addition,

wherever possible, the person conducting the measurements will be “blinded” i.e will not know what treatment the animals has received.

Refinement

Explain the choice of animals 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.

Refinement

There is no natural animal model for CF, but a mouse model has been generated using standard gene knockout technologies. The cystic fibrosis knockout mouse can suffer from intestinal problems. However, we will use a “gut-corrected” CF knockout mouse which has the CF ion transport abnormalities in the nose, but is otherwise healthy and normal. The mice do not have the characteristic CF gut disease, are not runted and do not suffer from increased mortality.

There is a naturally occurring haemophilia A dog model, but this model is unsuitable for early phase research. Haemophilia knockout mice are available and will be used for part of this research.

ADAMTS13 knockout mice are available with a range of genetic backgrounds. For this investigation the mildest genetic background and method for TTP induction has been selected in order to reduce suffering whilst still being able to gain meaningful and clinically relevant data. Mice will be humanely killed if, or as soon as treatment is seen to be ineffective in order to reduce unnecessary suffering.

Wherever possible we will use non-genetically modified mice.

Animals will be closely monitored following procedures and killed immediately using a humane method if undue suffering is likely and cannot be prevented by veterinary intervention. Procedures will be carried out under anaesthesia wherever feasible.

Wherever possible, we will be using in vivo bioluminescent imaging, a technique that allows repeated assessment on gene transfer in the same animal over time. This will reduce the number of mice required.

PROJECT 42

NON-TECHNICAL SUMMARY (NTS)

Project Title	Characterisation of anti-inflammatory virulence determinants;- role in bacterial persistence, gastric cancer and secondary infections.
Key Words	Immunosuppression, H. pylori;, S. Typhimurium, Microbiome
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

H. pylori and *S.* Typhimurium are bacterial pathogens that cause chronic infections by suppressing host immune responses. Here, we focus on a family of bacterial proteins, common to both *H. pylori* and *S.* Typhimurium, to understand how they suppress certain cells of the immune system, thereby facilitating chronic infection. Furthermore, we aim to study how *H. pylori* infection in the stomach affects the diversity of bacterial species in the intestine (by analysing faeces) to understand the mechanisms underlying the epidemiological link between *H. pylori* infection and protection against intestinal diseases, such as IBD.

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)?

Studying bacterial proteins, which are able to suppress certain cells of the immune system, may reveal novel drug targets for the treatment of chronic bacterial infections. Furthermore, understanding the mechanism of how these proteins stimulate anti-inflammatory signals from dendritic cells and/or T cells, will reveal novel structural determinants which can mediate immunosuppression. This may provide the rationale for drug design of novel anti-inflammatory therapeutics, which can be used to tackle auto-immune disorders such as asthma and IBD.

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

All of the studies proposed in this licence will use mice as the chosen model. The numbers of mice to be used in the next 5 years will be approximately be between 2500

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?

For immunisation studies, the most severe adverse effect is anticipated to be a local inflammatory response to the immunised antigen of interest, only expected to reach a moderate level of severity. After the immunisation, mice will be subjected to schedule 1 and organs harvested for further study. For H. pylori infection, no expected morbidity or mortality is expected in response to the infection. These experiments are only likely to lead to a very moderate level of severity. Mice will be terminated at relevant time points via a schedule 1 method and organs harvested for further study. For S. Typhimurium infections, mice are carefully monitored for signs

of infection as per the protocol above. The most severe clinical sign will be a weight loss of more than or equal to 20% of their starting weight, at which point mice will be humanely killed. Mice are only expected to reach a moderate level of severity for these experiments. After schedule 1, organs are harvested for further study

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In vivo, adaptive immune responses to bacterial infection develop through the interaction of multiple cell types including one or more DC subsets, T cells and B cells. We, and others, have repeatedly shown that these interactions in vivo are not fully replicated in vitro. Furthermore, we concurrently assess the response to the same antigen in multiple sites of the mouse (e.g. stomach, intestine, spleen, MLN and lungs). This is not possible in vitro nor in lower order animals (they lack an adaptive immune system) and therefore such approaches are unsuitable for this project.

In order to study the gastric mucosal immune response to the infection, and how it influences the community of bacteria in the intestine, we require intact gastrointestinal systems. Mice provide an established and verified means of studying immunity with tractable systems that allow detailed analyses of these complex environments.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Each experiment requires a written protocol giving full details of the experimental aims, a description of each group, including numbers, treatments and possible risks associated with the procedures used. This allows others to share experiment tissues etc post-mortem, reducing experimental numbers or permitting use of the same experiment to answer multiple objectives. For instance, after experiments are concluded, tissues such as the spleen are often sectioned for immunohistology and archived. This archived tissue can be revisited by other workers at a later time.

Refinement

Explain the choice of animals 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.

Refinement

Mice are an appropriate model as their immune systems share many similarities to those of humans including lymphoid organization and cellular populations (e.g. lymphocytes, DC). GA mice will be used throughout this project and provide a well-established means to study the immune system. Our experimental protocols are well established and published and have been carefully developed to identify the earliest time-points after the lowest bacterial dose to give biological meaningful results, with the minimal number of interventions (injections etc). For new protocols we work closely with[REDACTED] to refine the techniques to minimise any suffering that might otherwise occur.

PROJECT 43

NON-TECHNICAL SUMMARY (NTS)

Project Title	Cellular basis of neurodevelopmental disorders
Key Words	Synapse, neurodevelopmental, autism, brain, intellectual disability
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Developmental disorders of the brain (neurodevelopmental disorders) are often accompanied by autism, intellectual disability (ID), and epilepsy. These disruptions can be severely limiting, and there is an increasing diagnosis of neurodevelopmental disorders, autism and ID in the UK population.

The objectives of this project are to identify biochemical pathways that are disrupted in the brains of animal models of neurodevelopmental disorders, and to test new treatments to correct these disruptions. [REDACTED] has shown that the translation of genes into new proteins (protein synthesis) is abnormal in the brain cells of mouse models of autism and ID. Much of this research has been done on animal models of fragile X syndrome (FXS), the most common single-gene cause of ID and autism. Abnormalities in signalling pathways that regulate protein synthesis (i.e., the ERK and mTOR pathways) seem to contribute to learning deficits and seizures in these animal models. Importantly, there is evidence that drugs which target these pathways can correct problems in animal models, and some have shown promise in trails with FXS patients.

This project will look at new ways to target the signalling pathways controlling protein synthesis, and find new targets for treatment by further investigating the biochemical disruptions seen in animal models of FXS and related disorders. We are particularly interested in identifying signalling mechanisms disrupted downstream of NMDA-type glutamate receptors.

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

The major benefit of this research is the identification of new drug treatments for patients with autism-associated neurodevelopmental disorders such as FXS. [REDACTED]identified novel therapeutics that target key biochemical disruptions in animal models of FXS and related disorders, and these are currently being investigated in clinical trials. The goal of this project is to identify new and potentially better treatments for patients with FXS and other autism-associated neurodevelopmental disorders. The prevalence of autism and ID in the UK is approximately 1%, and thus the number of people benefitting from this work is significant. This research will also contribute to our knowledge of how the brain functions at the cellular level, which will be beneficial for the neuroscience research community.

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

It is estimated that approximately 15,000 mice and 5,000 rats will be required over the 5 years of this licence.

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

We will breed animals with disruptions in genes that have been linked to neurodevelopmental disorders. We do not expect these animals to suffer any adverse effects from these genetic alterations. The animals will be killed by humane methods and their tissues used after their death to study biochemical and electrophysiological changes of interest. In some cases animals may be given drugs to study the effects on biochemical/electrophysiological changes but it is not expected that this will cause any adverse effects other than mild clinical signs (such as decreased appetite or lower levels of activity). In some cases, we will test animals for their performance on learning and memory tasks or test them for audiogenic seizures. This will be necessary to reveal potential benefits from our drug treatments. Some of the learning tasks will involve giving a food reward to the animals, and they should not experience any adverse effects other than mild clinical signs. Other learning tasks will involve inducing a memory by delivering a mild foot shock, which may cause moderate discomfort from the stress involved in the delivery of the footshock. In cases where seizures are tested, we will expose the animals to a loud sound for up to 2 minutes, which may cause a moderate amount of discomfort. In all testing, attempts will be made to minimize discomfort to the animals (i.e., using the minimum loudness of sound to reveal seizures), and they will be humanely killed at the end of the experiment.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

This project requires biochemical and electrophysiological study of the mammalian brain, as well as investigation of complex behaviours. To date, experimental systems that allow this are limited and must involve the use of vertebrate animals.

Most instructive information about the mechanisms of synaptic plasticity and learning in neurodevelopmental disorders has come from studies of the intact nervous system. While reduced in vitro preparations such as cell line and neuron cultures can be used to assess certain aspects of cellular function, these do not faithfully reflect the intracellular signalling and electrophysiological properties of an intact circuit. Hence, the changes observed in those systems would be difficult to relate to the behavioural changes we are assessing in mouse models of neurodevelopmental

disorders. However, we will always use pre-existing RNA-seq/proteomics databases to investigate potential molecular/protein expression changes before performing new experiments using animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The proposed experiments will use the minimal number of animals needed to produce robust, reliable information. Experiments will be performed according to the ARRIVE guidelines. As such, genetically altered animals will always be tested alongside unaltered wild type animals that are matched for age and gender, and treated groups will always be compared to vehicle controls. Furthermore, experiments will be performed blind to genotype by using a code (e.g., A or B) to identify each animal. This code will be kept by a person who is not involved in the experiment. Groups will be assigned in a randomised fashion.

Power analysis calculations will guide the necessary group sizes for all experiments. For example, a power analysis (G*Power3.1 software) of previous data from a group of WT and genetically altered mice used for a protein synthesis experiment can be calculated as follows: WT mean=100, SD=16; KO mean=119, SD=21; Effect size=1.02; If alpha=0.05, Group size for 80% power=17. Given these numbers, a sample size of at least 17 will be required to find statistically significant effects.

For experiments involving brain tissue and brain slices, we will use material from one animal for multiple experiments after the animal's death. In many cases, two researchers prepare hippocampal slices from the same animal to perform their experiments. In addition, multiple researchers use the same brain lysate samples to perform Western blot experiments for proteins of interest. Nonetheless, this project requires the maintenance of a significant number of animals because (1) multiple behavioural and electrophysiological analyses will be performed, and each will require a *different cohort* of animals; (2) each of these experiments will require the use of at least 4 separate groups (i.e., WT vehicle, *GA* vehicle, WT drug and *GA* drug); (3) as some genes of interest are carried on the X chromosome, it would not be possible to compare female GA mice to female WT littermates. Thus, only male mice could be used for these experiments; and (4) according to power analyses performed on previous data sets, group sizes of at least 15-20 will likely be necessary for all of the proposed experiments.

Refinement

Explain the choice of animals 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.

Refinement

A major goal of this research is to identify and correct neurological processes that are disrupted in neurodevelopmental disorders using pharmacological and genetic strategies. The use of the genetically altered mutant rodent models is critical to investigating this question, because they effectively model human mutations that affect brain function. In the majority of our studies, we will use genetically altered mice in order to examine an intact mammalian nervous system. However, we will also need to use genetically altered rat models because they will allow us to assess more complicated cognitive disruptions that are difficult or impossible to observe in mouse models. For example, in behavioural paradigms, rats are more flexible in response to novel situations and have extensive social interactions - two domains specifically affected in autism spectrum disorders. This is particularly true of behaviours that depend on the prefrontal cortex (PFC), which is better developed in the rat brain.

The animals in these studies will be cared for by trained staff within a well-resourced and well-equipped modern animal facility that contains individually ventilated cages (IVCs) and barrier systems to maintain specific pathogen-free (SPF) status/health. Environmental enrichment will be provided.

All animals will be carefully monitored in the course of the experiments and if there is any evidence of suffering greater than minor and transient or in any way compromises normal behaviour they will be culled using schedule 1 methods.

PROJECT 44

NON-TECHNICAL SUMMARY (NTS)

Project Title	Zebrafish models of haematopoietic diseases
Key Words	Zebrafish models of haematopoietic disease
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The goal of this project is to develop new models of blood diseases (Diamond-Blackfan anaemia (DBA), myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL)), to further our understanding of these conditions and rapidly identify much needed new treatments. Most children born with DBA require life-long treatment and have an increased likelihood of developing cancer. Blood cancers we are studying in this project affect more than 5000 people in the UK each year and the mortality in adults remains extremely high (around 50% will die within 5 years). Furthermore in children, the treatment currently available result in life long toxicity. Better understanding and new treatments for these conditions are desperately needed.

To achieve the goals of this project we will need to breed and generate zebrafish that develop diseases that resemble the disease in people with DBA, MDS and leukaemia. We will use these fish to study their developing blood system and the onset of any tumours they develop. We will also use these models to identify novel treatments for DBA, MDS or leukaemia by exposing the zebrafish to libraries of different types of chemicals.

We have chosen to use zebrafish because we can utilise zebrafish embryos to screen for chemicals that may be of therapeutic benefit in children and adults with DBA, MDS or leukaemia and this is not achievable in any other living organism . Each zebrafish pair produces up to 300 embryos per mating, and they have fully functional blood system by 5 days of life. They are also transparent allowing us to observe the effects of many compounds in the live animals that model these diseases before they reach 5 days. There is no suitable cell culture system that would allow this type of analysis in vitro. Cells from patients with these conditions or volunteers will be used to confirm findings that we identify in zebrafish.

This project will provide valuable new zebrafish models allowing us to perform in vivo screens to identify new treatments of DBA, MDS and leukaemia. Drugs and mechanisms of disease identified from this project will improve our basic understanding of disease biology and may even identify new therapeutic treatments which could be used to treat patients in the foreseeable future. This is truly a realistic goal to aim towards since chemical screens in zebrafish have already led to a clinical trial of new drugs in patients receiving bone marrow transplants within 5 years of undertaking a screen such as that described in this project.

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)?

Developing effective animal models for specific blood disorders is crucial to understanding the disease. Better understanding of the genes that lead to blood diseases and progress in our ability to alter and track changes caused by loss of these genes permit us to study disease in a more targeted way. In the recent past, trialling new therapeutic molecules on zebrafish has sped up the process of taking drugs to clinical trials considerably. The nature of the zebrafish as a model organism, particularly in regard to its rapid development and quantity of embryos produced per breeding pair, allows for a large cohort of drugs to be trialled in a short space of time compared to other models.

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

All the studies described in this project are undertaken on zebrafish. The majority of studies will be undertaken on animals before the onset of independent feeding during which time we do not believe they are able to experience discomfort. These studies account for 8000 embryos per year. We expect that around 2200 adults per year will undergo procedures, however the vast majority of these are breeding procedures of animals that carry genetic modifications. Only 200 adult animals per year will undergo procedures that may result in moderate harm to the animal.

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

The majority of scientific procedures to be carried out on zebrafish are expected to have no or mild effects on the animals because they are predominantly conducted prior to the onset of independent feeding or under anaesthesia. Our goal is to develop fish that develop diseases akin to those we see in humans. This is the maximum amount of suffering we expect an animal to endure in this project is moderate severity, this accounts for 200 adult fish in this project per year. However this is the maximum amount of suffering we would anticipate. The actual number of animals experiencing moderate suffering in the context of what we are doing is around 10% of the animals undergoing these procedures (i.e. 20 fish per year). An example of this would be that a fish may develop leukaemia as a result of loss of a leukaemia gene and we may then treat that animal with a drug to assess whether this improved the disease in that fish. Both the leukaemia and the drug may result in some discomfort to the fish. Therefore they may experience adverse moderate effects from this such as difficulty with energy levels/swimming (due to anaemia or leukaemia). In the unlikely event that any animals show evidence that they have experienced moderate harm they will immediately be killed using a licensed technique that has been approved as being the most humane.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Where possible non-protected fish under 5 days of age post-fertilisation or primary samples from patients will be used in the described project. We have consulted web-based source to determine possible alternative strategies. Cell lines are unsuitable for the work described because they carry a large number of mutations and thus are likely to provide unreliable data.

We have also pre-screened substances where possible to show that small molecules are unlikely to cause harm to fish before we use them.

Some of the conditions we are modeling are caused by an abnormality in a single gene (e.g. Diamond-Blackfan Anaemia, DBA) . Importantly in this instance it is extremely difficult to use patient material for experiments as most of them are children, and the cells do not grow in the laboratory.

For our leukaemia studies our goal is to assess the biology and the effects of drugs on fish carrying genetic alterations that predispose to or "drive" the development of leukaemia. This is important because although a leukaemia may have many mutations, they remain dependent of the presence of one or two specific “driver” mutations that we are trying to target directly for new therapeutics. Therefore using this very targeted screening approach that assesses the effects of drugs in the whole animal provides an extremely rapid and specific method to find new drugs.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The choice of zebrafish is because it is very easy to follow the effects of disease and treatment with minimally invasive protocols. The animals are transparent during development permitting us to visualise effects on blood development prior to the onset of independent feeding. In order to study the development of leukaemias we have a large number of genetically distinct lines of fish permitting us to study the effects of individual genes and the interactions of several leukaemia causing genes in combinations that are observed in patients. This means that our research involves the maintenance of large numbers of adult fish. The majority of these fish are maintained solely for the use in natural matings to produce eggs for our research projects. Several factors determine the number of adult fish we need to maintain. The first is that all of the genetically distinct lines of fish need to be maintained as separate stocks. The different lines may contain mutations in different genes that are being analysed or may contain transgenes (such as green fluorescent protein) that allow us to track cells or manipulate gene function.

The second is to allow us to maintain fertility in this short-lived species. The third factor determining the numbers of adult fish that we maintain is the research demands placed on the specific line. E.g. those used for screening will need to be bred more often to obtain sufficient eggs and therefore more adults will be required.

To reduce numbers of animals used, our fish stocks are made available to other scientific personnel in order that only the necessary number of animals are maintained for use in procedures at UCL fish facility (providing experiments are covered by appropriate personal and project licenses). In keeping with this, great efforts are invested in maintaining all adult stocks in peak breeding condition. This ensures that we can keep the minimum numbers of fish required for embryo production. To facilitate this, all stocks are well documented on our databases with periodic assessment each month and detailed stocktaking performed by all personal licence holders every three months. These procedures help to ensure that we only maintain those fish required for our experimental research. In addition some projects may be more active than others at certain times and we regularly assess the need for maintaining lines and where possible store sperm for genetic mutations that we are not actively using fish from.

This project also utilises a new way of rapidly generating animals with mutations that minimises animal numbers by testing non-protected fish under 5 days of age post-fertilisation.

We will also reduce animal numbers by employing transplantation methods to test the effects of small molecules on leukaemia. In this way we will reduce the need to generate transgenic or mutant animals that may or may not develop leukaemia and may be more difficult to assess outcome of drug treatment.

Finally the work we have done validating human disease models in zebrafish will continue to reduce the number of mammalian models needed to validate biological findings or drugs.

Refinement

Explain the choice of animals 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.

Refinement

Zebrafish are the ideal choice of species for this project because our aim is to utilise zebrafish non-protected embryos prior to 5 days post fertilisation to screen for chemicals that may be of therapeutic benefit in children and adults with DBA, MDS or leukaemia is not achievable in any other vertebrate. Furthermore, while in some instances mice are preferred because they more closely resemble human disease, this is not the case for Diamond-blackfan anaemia and leukaemia also reflect the

diseases seen in humans. We have refined the way in which we house single fish that need to be isolated to track the development of disease by utilising new equipment that permits single fish to be housed and fed while remaining on constant water flow of our water system. In addition to continual water replenishment these transparent chambers permit fish to visualise many other fish in the system simulating social interactions that are preferred by this species.

PROJECT 45

NON-TECHNICAL SUMMARY (NTS)

Project Title	Development of humanised mouse models for study of cancer immunotherapy
Key Words	Transplantation, Cancer, Treatment, Immunotherapy, Safety
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

Cancer is a leading cause of death in the UK and most developed countries. The aim of this project is to refine and optimise novel models for the study of cancer cells and their therapy. The study will specifically investigate the interaction of the immune system with cancer cells and how the immune system can be manipulated to eradicate cancer cells in a specific and safe manner.

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 data generated by this study is essential for conducting human clinical trials on novel therapeutic agents for cancer. It is anticipated that this programme of work will validate innovative models for the study of cancer in the laboratory, which will enable more efficient and patient-specific screening of anticancer therapies. It is also anticipated that this programme will directly inform optimisation of novel anti-tumour therapies that can eradicate tumour cells specifically and with improved patient side effects.

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

Up to 8,900 mice will be used over a period of 5 years. Up to 775 rats may also be used during this period.

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

The majority (>80%) of the animals are not expected to show signs of adverse effects that impact materially on their general well-being. No more than 20% of animals are expected to show significant clinical signs as a result of the effects of irradiation and restoration of the immune system, surgery or treatment with drugs. Rarely the severity of these signs may be such that the humane end points may be reached. Mice will be killed if they show signs of ill health, such as weight loss, piloerection and hunched posture or inactivity. All animals will be humanely killed at the end of the experiments.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The definitive examination of the efficacy and safety of anti-cancer drugs requires examination in intact animals, including those with a competent immune system. This is a necessary and pre-requisite ‘final’ step for the clinical translation of these cancer therapies and cannot be completed without animal experiments. Much of the proposed work is carried in the laboratory and using human tissue only, thus minimising the need for animal experimentation. Importantly, it is anticipated that this work will lead to the refinement and optimisation of laboratory models of cancer which can be ultimately used to replace experimental use of animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Only tumour models and therapies that are supported by compelling laboratory experimental data will be investigated using animals. The studies are designed such that many groups of animals will generate valuable data pertaining to tumour biology as well as the efficacy and safety of therapies. Furthermore, some animals serve as controls for more than one experimental group, whereas in other experiments, the same animal can be used as its own control (for example, by being transplanted by two types of cells). Randomisation and blinding will be used to minimise bias and the total number of animals are significantly reduced by addressing all aims using the sophisticated experimental design utilised in this project.

Refinement

Explain the choice of animals 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.

Refinement

The models used are optimally suited to achieve the aims and objectives of the study. We have refined the protocols and procedures for the generation and maintenance of these mice to maximise the likelihood of the success of the experiments and to minimise stress and harm to animals. The vast majority of the experiments are designed such that the animals only experience minor discomfort, and serious ill health or death is never an expected end-point.

PROJECT 46

NON-TECHNICAL SUMMARY (NTS)

Project Title	Understanding and treating developmental disorders
Key Words	developmental disorders, childhood, disease mechanism, behaviour
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Over 20,000 children are born each year in the U.K. with developmental disorders such as autism spectrum and rare intellectual disability disorders. For most, the cause is unknown. Even when the genetic cause is known, treatments are rarely available for these diseases. We aim to identify previously unknown causes of childhood disorders, understand how damaged genes cause these disorders, and test whether some might be treatable with medicines.

We aim to find which disorders can be treated, and communicate these findings to the wider community, to encourage others to develop treatments for human patients.

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)?

Uncovering new genetic causes for developmental disorders will increase our ability to diagnose these children, and provide better prediction for how a disorder might progress, both leading to better patient care. Knowing the cause can also increase interaction between patients with the same disorder (through patient networks), thus improving quality of life. Understanding how gene changes cause the disorder can give fundamental knowledge leading to better care and treatment. Testing whether some disorders are treatable, and which drugs might be effective, will provide the first steps towards potential treatments for specific disorders. This should lead to the development of treatments for patients with these disorders.

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

Over a period of 5 years, we will use up to 25,100 mice for breeding. We will use up to 7,500 mice for experimental procedures.

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

The majority of animals used in these experiments will experience enriched, novel environments throughout the testing period, which could result in mild and transient anxiety when first introduced. Some animals, due to the genetic changes made to mimic those seen in patients, may experience mild symptoms such as memory problems, learning difficulties, and decreased sociability. Animals treated with medicines may have side effects like loss of weight. The animals will be closely monitored to look out for any adverse effect to avoid animal suffering. Some experiments may result in mild, temporary weight loss. At the end of the studies, the mice will all be killed using humane methods. Samples may be collected from them for measuring the body's response to the gene changes they carry.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Developmental disorders occur during the growth of the animal, and are by definition a feature of multicellular organisms with specialised tissues. The outcome of these disorders is typically a change in learning or behaviour. Using cells in a dish cannot adequately reproduce this complexity, nor that of the brain. There are currently no non-animal models that can capture this complexity without sacrificing the essential concordance with the human disease. For this reason, it is necessary to use animals for these experiments.

However, in parallel studies we are using human cells to try to replicate some of what we see in animal models. If successful, this may lead to a future replacement of animal experiments with ones using cells in a dish.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Whenever possible we intend to use mouse colonies that already exist, using national and international repositories rather than create them ourselves. When new colonies are needed, we intend to use a service that has specialist expertise, which will reduce the number of animals used for generation.

The work is also carried out in a facility with strict environmental controls, so we reduce the variation in our mice and therefore require fewer animals to find behavioural differences.

We intend to carefully select the human disorders we will model, selecting ones that are caused by a single copy of a mutated gene, and strong evidence that they cause disease. This will mean we need to breed fewer animals to obtain enough of the right type for our experiments, and are more likely to have a meaningful result.

By using previously published results that employed the same tests, we will carefully plan to use the minimum number of animals sufficient to give us a statistically meaningful result.

By sharing our data results with other researchers, we will avoid duplicated efforts and reduce number of animals used globally. We will publish our findings in open access journals to share results freely and publicly.

Refinement

Explain the choice of animals 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.

Refinement

Mice are a suitable species for this project because they have similar brains and sensory systems to humans and display many of the same behaviours. Mice also have very similar genomes to humans, so the majority of human genes we would like to study exist in mouse. Mice are good at learning new tasks, so we can quickly train them and detect any problems in learning due to the changes we make.

Because we want to be able to turn genes back on in adult animals, mice are uniquely suited to these types of genetic manipulation through decades of related research in mouse.

Mice are social animals, so in this project they will be group housed, except for specific periods where single housing is required for scientific reasons. The mice are provided with cardboard tunnels and nesting materials to facilitate normal behaviours. The use of fun tunnels when handling will reduce the stress to the animals when they are picked up or moved.

Some mice will be housed in special cages that automatically record their behaviour. They will benefit from reduced handling.

When we use young animals, extra care will be taken to ensure maternal attention. When drug treatments are undertaken, we will investigate what is already known about the specific treatments in order to maximise beneficial outcomes. To maintain the wellbeing of treated young mice, we may on occasion treat the mother, who will pass the medicine through her milk to her pups.

We use a sophisticated database system for tracking the tests the mice have had and for monitoring of health and welfare concerns. This allows live reporting on the condition of every mouse, so that if a mouse is found to have a health problem, swift decisions can be made about its welfare.

By considering size and age of animals, we will reduce the risk of fighting during the pairing of non-familiar animals.

PROJECT 47

NON-TECHNICAL SUMMARY (NTS)

Project Title	Pathogenesis and prevention of infections by respiratory pathogens
Key Words	respiratory infection, bacterial pathogen, vaccine, Streptococcus pneumoniae, pneumonia
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

This project will use mouse models of infection to investigate lung infections caused by respiratory pathogens such as the main cause of pneumonia, *Streptococcus pneumoniae*. The ultimate aim is to identify novel ways of treating or preventing these infections. Lung infections are a major clinical problem worldwide – they are the commonest cause of death due to bacteria, and one of the major causes of death in children under 5. They are also very common in the UK, with pneumonia causing about 65000 deaths per year. Pneumonia is particualrly common in the elderly affecting about 1 in every 100 person over the age of 75 each year. However at present we do not have good vaccines that prevent the common bacterial causes of pneumonia in adults, and resistance to antibiotics is becoming commoner amongst the bacteria that cause lung infections. It is not clear why some bacteria such as *S. pneumoniae* often cause pneumonia whereas other closely related bacteria such as *Streptococcus mitis* do not. This project will further our understanding of what allows severe infections with respiratory pathogens to develop and how they can be prevented, and will eventually lead to improved preventative (including vaccines) or treatment strategies against these pathogens.

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

1. Improved understanding of why and how serious lung infections can occur 2. Identification of potential targets for new antibiotics 3. Further development of new vaccines that are able to prevent respiratory tract infections or pneumonia

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

Solely mice - we will use between 200 to 500 mice per year over the 5 years of the project.

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?

Mice will be infected usually via inhaling micro-organisms under general anaesthetic or by injection into the blood or peritoneal cavity. Depending on the experiment the mice may have been vaccinated with potential vaccine candidates or treated with a drug therapy prior to or after infection. In the majority of experiments, mice will then be culled before severe infection develops and the response to infection analysed in target organs such as the blood, lungs and spleen. These timepoint experiments

only require small numbers of mice per group (5 to 10) and, because mice are culled before severe infection develops, minimise any distress caused. Rare experiments will monitor whether a vaccine, bacterial mutant, or a drug effects the development of infection over time by watching for physical signs that infected mice have developed severe infection. The main adverse effects of these experiments are failure to recover from a general anaesthesia, local discomfort and very rarely significant trauma necessitating immediate culling after injection, and the signs and symptoms of infection (weight loss, piloerection, reduced mobility, and depending on infection site possibly cough, respiratory distress, diarrhoea, local tenderness, erythema and swelling). All animals are culled humanely at the end of the experiment using a schedule one method or exsanguination (terminal bleed from the heart under deep terminal anaesthesia).

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The incredibly complexity of lung anatomy, immune responses to infection, and bacterial / host cell interactions prevent these experiments from being done without using animal models of infection. For example, the lungs have a three dimensional structure consisting of a mucosal layer with multiple cell types, and during infection several different types of white cells are recruited to the lungs with their relative proportions varying in a very dynamic way. A vaccine will alter the response to the infection in multiple ways. In addition the bacterial infection may spread from the lungs elsewhere within the body. This highly complex process can not be fully replicated in laboratory cell culture models, nor by insect or fish infection models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We minimise the number of animals needed by:

1. Doing laboratory tests using models of specific bacterial host interactions to fully define how a given bacterial mutant or component interacts with the host before moving to animal models - that way we can be very specific about the information needed from an animal model and thereby reduce the number of mice required. Recently we have expanded the laboratory testing to use more human cells and even slices of human lung to further refine the data we may need from animal experimentation.

2. By only looking for large biological effects using animal models - these need fewer mice than subtle effects to obtain a statistically significant result.

3. For the majority of experiments using infection models that can lead to important data with small numbers of mice eg competitive infection experiments that only need 3 to 5 mice, and timepoint experiments that need 5 to 8 mice per group.

4. Using technical developments that means we can monitor bacterial numbers in the same mice repeatedly over time i.e. using nasal presses to assess bacterial colonisation of the upper respiratory tract, or imaging of fluorescent bacteria in live animals for infections affecting the lung or blood.

Refinement

Explain the choice of animals 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.

Refinement

Our experiments are performed in mice as infection in mice usually closely mimics human infection and because genetically modified mice provide a powerful tool for identifying important host factors involved in the development of infection. We minimise welfare costs of the infection experiments by: (a) using for the majority of the infection experiments pre-selected timepoints for culling mice – this means that most mice will not develop severe evidence of disease before being culled; and (b) close monitoring of mice over the experimental period to identify any that may develop evidence of unexpected

PROJECT 48

NON-TECHNICAL SUMMARY (NTS)

Project Title	Cancer Drug Discovery
Key Words	Cancer, Immunology, Therapy
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Over 150,000 people die from cancer in the UK each year. Although there are have been significant advances in recent years it is clear early there remains an urgent need for more effective treatments as well as for treatments with fewer negative side effects.

Recent high profile successes have come from “immunotherapy”, which seeks to harness the body’s immune cells to better fight and hopefully eradicate the cancerous cells. Despite these successes, the new drugs are far from 100% effective and have considerable side effects. Nevertheless they have prompted an intense global focus of pharmaceutical companies on designing new and better immunotherapies for cancer.

Our main objective is to contribute to this field of knowledge by identifying novel substances that can be used in immunotherapy against a range of types of 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)?

There are many aspects of the anti-cancer immune response that can be potentially boosted to develop new immune based treatments for various types of cancer, for example pancreatic or breast cancer. This requires an expert basic knowledge of the immune system which is our area of expertise. We provide a wide range of sophisticated cell and animal based models which can monitor the effects of new drugs on the immune system and identify those with potentially anti-cancer activity. Work with cells in the laboratory makes up a significant proportion of what we do and the first steps in identifying potential new treatments do not involve live animals. This is making the process of developing new drugs faster and more efficient. So the overall aim is to more rapidly and effectively identify anti-cancer drugs that can progress into human trials. These studies will benefit the scientific and pharmaceutical community but ultimately the goal is to improve treatments for patients with a range of cancer diagnoses where treatments are currently unavailable or not effective. It is likely in the longer term that these treatments may also benefit the veterinary field in providing novel treatments for animal cancers too.

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

We will use mouse immune and cancer models that are already established and used widely in cancer studies. We anticipate that we will use up to 5,000 mice in cancer studies and up to a further 3,000 in more simple immunology studies, 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 levels of severity? What will happen to the animals at the end?

Broadly, the cancer models we will use come in two forms. Some mice are modified to have gene mutations that are known to drive different forms of human cancer. In these models the cancer can therefore arise spontaneously (or after administration of an inducing chemical). In the other type of model, cancer cells are implanted or injected and grow, either in the organ from which they were derived, or under the skin. In general, the mice do not show ill health as a result of the cancers themselves, because our experiments do not need run to that stage. In the simpler immunology experiments, the mice receive one or more injections, which may cause some localised inflammation. Some studies do require that the cancer model is allowed to develop for a longer period of time in order to mimic what would happen in human disease. In this scenario animals are very closely monitored and will be killed if clinical signs are seen beyond that allowed by the project licence.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Importantly, many of our assays are performed in the laboratory, rather than in animals. These often use human immune cells, including from cancer patients. This allows us to test how different immune cells respond to potential drugs. However, once we have defined the most likely candidate drugs, we must understand whether these can in fact help the immune system to fight the growth of cancers in the body, which is a much more complex requirement than can be modelled in the laboratory.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Our advanced immunology assays allow us to advise our clients on which of their test compounds are most likely to be effective in cancer studies. This saves time and money and, most importantly, reduces the numbers of animals required for cancer studies.

Refinement

Explain the choice of animals 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.

Refinement

Mice have the best characterized immune system, with many reagents available, allowing us to perform detailed immunological studies. There is also a wide range of different cancer models, that mimic human cancers, available in mice. This is important because certain drugs might be expected to target particular forms of human cancer more effectively than others.

We have well defined end-points (size of tumours) in place, which determine when an experiment will end. The mice a carefully monitored for tumour growth and for their general health. In models that involve surgery, appropriate anaesthesia is given, as well as pain relief as required.

PROJECT 49

NON-TECHNICAL SUMMARY (NTS)

Project Title	Improving therapies for blood cancers
Key Words	Leukaemia, Extramedullary disease, Blood cancer, Lymphoma, Central nervous system
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Acute lymphoblastic leukaemia (ALL) is the commonest childhood cancer. Great advances in treatment have been made, so that now more than 90% of children survive. However, the impact of chemotherapy on children is significant and efforts are now being made to personalise therapy and develop kinder and gentler treatments.

Up until recently most clinical research has focussed on improving and refining treatments to eradicate leukaemia from its site of origin (the bone marrow). However, the brain (central nervous system, or CNS for short) and other organs (collectively called “extramedullary” (EM) sites) can also harbour leukaemic cells which, if inadequately treated, may cause leukaemic relapse (recurrence). Much less is known about the best ways to treat leukaemia in EM sites including the CNS.

Our laboratory has established expertise in extramedullary ALL. Our current objectives are:

1. To understand how leukaemic cells move to and survive in new environments such as the CNS (brain).

2. To use information from 1 to design and test better, less toxic therapies to prevent leukaemic relapse in EM sites such as the CNS.

3. To use our expertise derived from studying ALL to see if the same mechanisms are involved in EM spread of other blood cancers such as acute myeloid leukaemia, chronic myeloid leukaemia and lymphomas.

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 programme of work aims to identify new drug targets and therapeutic strategies that may be less toxic and more effective at eradicating ALL and other blood cancers, especially those that have spread to sites outside the bone marrow. This would be a significant advance as currently all children with ALL receive large amounts of CNS-targeted chemotherapy including lumbar punctures with direct injection of chemotherapy into the spinal fluid. This treatment is unpleasant and can cause side-effects including stroke-like episodes, seizures and problems with learning and memory.

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

Mice will be the only species of animal used. All experiments will be designed to employ the minimum number of animals that would achieve a meaningful result.

Over the course of this programme of work (5 years) we estimate we will use approximately 3500 mice.

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

The basic model for our studies requires leukaemia development in the mouse following injection of human leukaemia cells. We will use the least invasive methods for monitoring of leukaemia such as identifying circulating cells on small blood samples prior to the onset of disease symptoms (such as fatigue, loss of appetite and weight-loss). This will allow us to achieve our study aims with minimal animal suffering as we can intervene before the mouse develops other symptoms relating to leukaemia We will also maintain the wellbeing of our mice by administering antibiotics, avoiding unnecessary handling and minimising any discomfort experienced by using appropriate analgesia and/or anaesthesia during and after any procedures. Once we have achieved our experimental aims the mice will be humanely killed. Based on accumulated personal and worldwide experience with these models the maximum severity expected is moderate.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Our central aim is to understand how leukaemic cells adapt to different environments within the body. We are currently unable to recreate these complex biological environments (comprising multiple different cell types, supporting matrix, nutrients and blood supply) in test-tubes or tissue culture flasks. Since we need to study cells taken directly from different sites around the body we have two alternatives - animal models or patient samples. We use samples from patients whenever they are available and often use this information to focus down on specific questions or targets in our mouse models but patient material is often very limited and generally does not survive or grow once outside the body. Using mouse models, we can not only identify molecules responsible for leukaemic spread or therapy resistance but also modify the leukaemia cells and/or test new drugs to see if this can be overcome. These are essential pre-requisites to developing new clinic-ready therapies for patients with leukaemia and other blood cancers.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will design experiments to use the minimum number of mice to achieve valid results by use of power calculations and other appropriate statistical modelling. Such planning will minimise the number of repeat experiments required to confirm results. Where possible we will optimise experiments using alternative models such as drug dosing on cell lines and only take promising candidates through to animal studies.

Refinement

Explain the choice of animals 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.

Refinement

Mouse models are known to closely mimic many of the characteristics and features of human leukaemias including infiltration of the CNS and other EM sites. There is significant similarity between mouse genes and proteins and their human counterparts. The mouse models that we propose in this plan of work are well established as highly informative models in which to study mechanisms of leukaemia development and survival and to test potential drug targets.

The need to keep the suffering of the mice to a minimum is always taken into account when planning the experiments. In each experiment mice will be observed very closely for adverse effects and steps will be taken to minimize pain or discomfort. Mice will be housed in cages with environmental enrichment and will be subject to sympathetic and humane care. Animal suffering will be kept to a minimum by the application of good experimental technique, use of anaesthetics and pain controlling drugs and careful monitoring so as to intervene prior to the occurrence of significant suffering.

PROJECT 50

NON-TECHNICAL SUMMARY (NTS)

Project Title	Drug evaluation in pre-clinical oncology models
Key Words	Cancer, pre-clinical, efficacy, models, imaging
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The primary objectives of this project are:
(i) the use of established and validated mouse models of cancer to evaluate candidate anti-cancer agents and combination therapies, to support progression of effective anti-cancer treatments to human trials, ultimately resulting in validated effective final products.
(ii) To support objective (i) through the development of patient relevant pre-clinical models for the evaluation of candidate anti-cancer agents and combination 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)?

According to studies published in the public domain, 77% of 800 cancer drugs entering early clinical trials failed to reach patients and this failure was attributed in the majority of cases to poor response to the anti-cancer agents in the patients tested. This data highlights the growing need by the Pharmaceutical, Biotech Industry and academia for more patient-relevant and predictive cancer modelling before clinical trials begin especially with new generation of targeted cancer drugs, immuno-therapeutics and combination treatments that are presenting new opportunities for patients with cancer. The aims of this project is to provide the scientific community with a high level of centralised expertise in terms of available clinically relevant cancer models, knowledge and technical capability to improve decision making on which agents should progress to the clinic and which patients will benefit from the treatment. In some cases this may result in programme cancellations; whilst this may seem of negative benefit, cancellation of candidate anticancer agents either ineffective or unsuitable for further development can be considered a positive benefit in the longer term as it limits the progression of ineffective therapies brought to early phase clinical trials and allows the direction of resources and patients to other projects. As the understanding around the mechanisms behind cancer progression continues to increase, so does the requirement to develop and validate relevant models in parallel to test new strategies. Thus the best way to benefit the scientific institutes that we work with, industry and thus patients as a whole, is by the development of pre-clinical cancer models that exhibit greater patient relevance for their application to the development and testing of novel anticancer agents. We are very proactive in attendance at relevant national and international scientific conferences and actively share our research where possible with the global scientific community through abstract submission to national and international conferences. Once validated, all models are added to the proprietary databases; access to which is free to all users, so one of the immediate benefits of the model development process is that model data, including

growth and response to standard therapies, histologic and genetic characterisation is freely available to the scientific community which makes these databases extremely powerful tools for research. What types and approximate numbers of animals do you expect to use and over what period of time? Mice will be used for the entirety of the project as they are the lowest species of animal that allow the modelling of human cancer, and offer the opportunity for genetic manipulation to generate specific models relevant to human cancer. Substantial numbers of cancer relevant models are already validated in-house (100+) making this species most amenable to this course of research to investigate different cancer types which include breast, prostate, lung, brain, bladder, leukaemia, lymphoma, multiple myeloma, colorectal, fibrosarcoma, gastric, head & neck, kidney, liver, thyroid, melanoma, oesophageal carcinoma, ovarian and pancreatic. Over the course of this project we'd expect to use 115,700 animals to model these cancer types, the different stages of cancer and novel anti-cancer agents and combinations.

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

Mice will be used for the entirety of the project as they are the lowest species of animal that allow the modelling of human cancer, and offer the opportunity for genetic manipulation to generate specific models relevant to human cancer. Substantial numbers of cancer relevant models are already validated in-house (100+) making this species most amenable to this course of research to investigate different cancer types which include breast, prostate, lung, brain, bladder, leukaemia, lymphoma, multiple myeloma, colorectal, fibrosarcoma, gastric, head & neck, kidney, liver, thyroid, melanoma, oesophageal carcinoma, ovarian and pancreatic. Over the course of this project we'd expect to use 115,700 animals to model these cancer types, the different stages of cancer and novel anti-cancer agents and combinations.

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

The mice used in this project will be used to support candidate anticancer agent development through the following stages/types of projects and model development: I. Pharmacokinetic testing: Mice will be dosed with candidate anticancer agents to determine the fate of a chemical from the moment that it is administered up to the point at which it is completely eliminated from the body. This information is used to guide dosing regimens to ensure sufficient agent is delivered for a sufficient period of time to achieve effective target efficacy (achieve mechanism of action) in later project stages. II. Pharmacodynamic (PD) testing: Mice will be dosed with candidate anticancer agents to generate information about the efficacy of the candidate anticancer agent against its tumour target. Taken in consideration with PK analysis they can be used to assess suitability for progression to efficacy testing. The majority of mice will undergo subcutaneous tumour implantation which are visible and measured by callipers (length and width); less commonly surgical tumour

implantation into the brain or organs such as liver/lung under anaesthesia which are then measured once/twice weekly throughout the study by imaging under anaesthesia to determine internal size. Once small tumours have established single doses of candidate anticancer agents by standard routes (oral, intravenous, subcutaneous etc.) will be administered followed by scheduled in life and terminal sampling. Tumour and tissue samples will be used to determine impact of agent/dose on modulation of tumour target. The short study/dosing duration and small tumour burden means that the prevalence of treatment-related adverse effects is uncommon in these studies, and any adverse clinical signs are expected to be transient. All mice will be killed at the end of the studies. III. Tolerability testing: The key aim is to ensure that candidate anti-cancer agents are tolerated at the proposed dose levels/regimens prior to entering into larger efficacy testing protocols. Mice will undergo minimally invasive procedures: short dosing phases (up to 2 weeks) at regimens reflective of follow-on efficacy studies by standard routes (oral, intravenous, subcutaneous etc.); occasionally in-life blood (tail or saphenous vein) sampling or terminal sampling is carried out. Care is made to select a dose regimen to minimise toxicity and informed by PK/PD studies; however, body weight loss (BWL) and/or adverse clinical signs may be evidenced as a result of acute or cumulative dosing. Body weight will be monitored daily and will be used to guide to intervention. Persistent adverse clinical signs e.g. subdued behaviour patterns even when provoked etc. will result in humane killing regardless of body weight measures. If the initial dosing regimen produces evident toxicity, doses will be reduced by a stepped approach (~30-50%) prior to testing in further tolerability studies. All mice will be killed at the end of the studies. IV. Subcutaneous (s.c.) efficacy testing: For s.c. efficacy testing, the key aim is to assess the efficacy of candidate anti-cancer agents, either as monotherapy or in combination with other candidate anti-cancer agents on the growth of mouse or human tumours. Mice will undergo subcutaneous tumour implantation by cancer cell injection or tissue implantation under anaesthesia. Dosing of candidate anticancer agents (refined through earlier work) by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response or humane endpoints for tumour size, mean diameter ≤15mm, are achieved. Provision of supporting tolerability data or acute phase tolerability studies (section E) means that the frequency of treatment-related adverse effects are uncommon in these studies; however, body weight will be monitored daily during dosing phases and will be used to guide to intervention. Persistent adverse clinical signs e.g. subdued behaviour patterns even when provoked, will result in human killing regardless of body weight measures. All mice will be humanely killed at the end of the studies. V. Efficacy studies with genetically modified mice which carry the same mutation to that in human colon cancer resulting in similar tumour formation and progression will be dosed with candidate anticancer agents, refined through earlier work, by standard routes until scientific endpoints are achieved i.e. development of adenomas in the small and large intestines by 18 weeks; Alternatively, a surrogate survival format may be employed using a humane

endpoint i.e. the onset of anaemia; in this setting, the study can be terminated at that point at which a statistically significant effect on surrogate survival can be determined. All mice will be humanely killed at the end of the studies. VI. Translational studies: experimental metastasis: Experimental metastasis models mimic latter stages of disease progression that may be difficult to model utilising spontaneous metastasis models where primary tumour size may drive the model endpoint. Mice will undergo tumour implantation by cancer cell injection (intraperitoneal, intracardiac, or intravenous). Dosing of candidate anticancer agents by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response (as assessed by optical imaging) or humane endpoints for tumour progression e.g. abdominal distension (peritoneal ascites), changes to gait (bone metastasis), or respiratory changes (lung metastasis.) Provision of supporting tolerability data or acute phase tolerability studies means that the frequency of treatment-related adverse effects are uncommon in these studies; however, body weight will be monitored daily during dosing phases and will be used to guide to intervention. Persistent adverse clinical signs will result in humane killing regardless of body weight measures. All mice will be humanely killed at the end of the studies. VII. Translational studies: Models implanted in relevant organ sites are known to better model cancer in patients with respect to various criteria as they form a single focal disease area as in the patient situation, facilitate metastatic spread via lymph nodes and show a reduced response to chemotherapy. Mice will undergo tumour implantation by cancer cell injection and surgical tumour implantation into the brain, lung or liver under anaesthesia. Dosing of candidate anticancer agents by standard routes will be administered until scientific endpoints i.e. the statistical comparison of treatment to control response (as assessed by optical imaging) or humane endpoints for tumour progression e.g. abdominal distension (peritoneal ascites), lack of coordination, head-tilt (brain tumour), or respiratory changes (lung tumour) Provision of supporting tolerability data or acute phase tolerability studies means that the frequency of treatment-related adverse effects are uncommon in these studies; however, body weight will be monitored daily during dosing phases and will be used to guide to intervention. Persistent adverse clinical signs will result in humane killing regardless of body weight measures. All mice will be killed at the end of the studies.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In vitro methodologies have replaced animal use in early development phases, particularly in the development of screening assays to refine compound selection,

target identification, off-target toxicity or toxicity versus normal tissue cell lines, and can certainly guide and refine the steps prior to moving into in vivo, and minimise subsequent use. However, there is still a requirement to use animals for this project as in vitro assays still do not optimally mimic all interactions between cells and tissues in vivo, such as blood vessel formation, spread to other organs and thereby relevant drug access or the many homeostatic mechanisms in play in an in vivo environment that allows relevant tumour biology drug evaluation.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The use of in vitro studies can be used to identify lead compounds, evaluate dose ranges confirming target modulation/expression and relative off-target toxicity which can be used to inform on relevant doses for use in PK, PD and pilot toxicity studies. The use of complex 3D in vitro assays can be applied to pre-screen studies and compound selection prior to advancement into animal testing (thus reducing animal use). Careful use of pilot studies and statistically powering the study design can be used to optimise animal model use and reduce overall use of animals. The use of optical imaging technologies can reduce the number of animals required to generate study outcomes.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the lowest species in which the knock out of the immune system allows growth of human tumours. Mice with a fully functioning immune system also provide the opportunity to investigate a large panel of mouse cancer models to enable the immune system interplay with the tumour to be investigated. Genetically engineered mice with mutations representing those seen in humans, for example in colon cancer, will also be used to assess the importance of potential oncogenes and mice are the lowest species in which this technology can be applied as require an appropriate mammalian architecture. There will be 2 main approaches to tumour implantation. The majority of mice will have tumours implanted subcutaneously as this enables immediate and accessible measurement of tumour growth for a wide range of cancer models. The second approach is to implant tumour at the site of origin which are more relevant to patients but are more complex and require imaging to track the growth inside the mouse.
Although subcutaneous cell line models lack direct translation to human studies, they

are extremely well characterised within the scientific community (peer-reviewed scientific literature). As such they can be a useful tool if used with acknowledgment of their limitations i.e. as a tool to help 'dissect' a specific molecular pathway, gene fusion or driver mutation. In this context they allow a flow of work from early in vitro studies, through to PK/PD and efficacy assessments, thus assessing proof-of-concept in a minimally invasive scenario. Furthermore, using optical imaging technology they can be translated to more complex organ-specific and metastatic modelling to offer a more translational context.

PDX models offer significant translational power, they preserve both the genomic integrity and heterogeneity of the original disease and allow the generation of data that closely resemble clinical data. The translational power of PDX models is increased with their application to pre-clinical Phase II-like mouse clinical trials (MCTs) that closely reflect the human trial design, studies can be used to inform on patient selection or dosing strategies in human trials.

Organ-specific models are known to better model cancer in patients as tumour grows in the correct environment which facilitates spread to other organs via the lymph nodes as seen in the clinic and also show a reduced response to chemotherapy. The use of optical imaging will be used to refine the methods used as well as minimise animal suffering, as it allows the opportunity for the determination of a statistically significant result ahead of a scheduled termination, thus potentially reducing the duration of regulated procedures.

Experimental metastasis models mimic latter stages of disease progression e.g. escape from primary site, establishment at the metastatic site, compartmental separation etc. that may be difficult to model utilising spontaneous metastasis models where primary tumour size may drive the model endpoint. Experimental metastasis models are therefore useful for assessing candidate anti-cancer agents directly targeting the development of metastasis, or metastatic treatment strategies which often differ to those used for primary disease in the clinic. In the case of intracardiac administration of cells (i.e. experimental bone metastasis), this results in a much more refined model than direct injections into the bone as the circulating cells encounter the target organ e.g. the capillary beds of the bones, in the same way as circulating metastatic tumour cells arising from a primary tumour. In the capillary beds they are compelled to invade into the tissue, thus only the clone of the cell population having the required capabilities e.g. tropism conferred by possession of the bone metastasis gene expression signature will survive and grow into a tumour. Direct injection into the bone introduces the cells directly into the site and does not model the escape of cells into the bone site. Furthermore, direct injection may result in the mechanical disruption of the bone itself, which is not only aversive to the animal, but could also compromise the development of lytic lesions that are characteristic of many breast and prostate bone metastases.

The development of relevant pre-clinical models of oncology is a key stage for the evaluation candidate anti-cancer agents and proposals for model development will undergo a review by the [REDACTED]internal research and development (R&D)

committee. Following completion of the model development phase, a report will be generated and the outcomes of the model development process will be carefully reviewed by the R&D committee before the model is considered suitable for use in client studies. As part of the ongoing commitment to the highest levels of scientific output and welfare, a regular review period will be set up for each model following completion of the model development process. This will look to follow-up on the current use and applications of the model to ensure that the most refined science and animal welfare is being utilised. Where areas of potential refinement are identified, these will be assessed through further pilot and validation ?studies.
in summary the methods that minimise animal suffering include the following:

- Pilot studies for the establishment of new tumour lines and refinements to surgical techniques will be carried out on an ongoing basis under the advice of the NVS/NACWO will be sought in this respect.
- All surgical procedures will be conducted in line with established welfare guidelines on aseptic surgery using suitable anaesthesia along with peri and post-operative analgesia.
- Presentation of adverse clinical signs, behaviour patterns or BWL relating to treatment or model progression should be de-risked by supporting work, and managed as detailed in the relevant project plan and protocol sections.
- Sampling will be in line with established welfare guidelines (see general project plan comments), and micro-sampling regimens will be utilised where study design supports this.
- The frequency of dosing will be such that animals fully recover between injections and will not suffer more than transient pain and distress and no lasting harm and there will be no cumulative effect from repeated injections.
- The use of supplemented diet or drinking water may be used for both candidate anti-cancer agents as well hormone supplementation, but in such circumstances, care should be taken to carefully monitor intake, to ensure that that the change in composition doesn’t affect normal feeding/drinking behaviour.
- For hormone dependent models (some oestrogen-dependent breast/ovarian models, and some androgen-dependent prostate models) hormone supplementation using the most refined method that results in consistent tumour growth.
- For test agents whose efficacy may be impaired by the blood brain barrier, small proof-of-concept pilot studies may be carried out whereby dosing is achieved by administration directly into the brain or tumour site. Where multiple doses are required use of an intracerebral/intraventricular cannula will be used to reduce the number of invasive procedures.
- Use of pilot tolerability studies to ensure there are no unexpected adverse effects associated with new models or unexpected toxicity as a result of tumour:drug interactions and to ensure the drug levels used are not associated with any cumulative effects.
- Mouse tumours implanted in mice with a fully functional immune system display higher levels of ulceration, therefore appropriate scoring system with defined

endpoints and escalated actions has been put in place as a refinement to these models, and ensures that the welfare of the animals isn't compromised and the risk of harm is minimised throughout the model but scientific endpoints are still achieved.

- Non-invasive imaging will be used to refine the methods for all orthotopic and metastatic cell line models as well as minimise animal suffering, as it allows the opportunity for the determination of a statistically significant result ahead of a scheduled termination, thus potentially reducing the duration and of regulated procedures as described in Section D general comments.
- During surgery where there is a need to go through the muscle wall local anaesthesia will be used as additional pain relief. Pain scoring will also be carried for 3 days post op. A standard approach to post-operative pain management is to provide analgesia for 3 days post op, by giving a NVS recommended analgesia in flavoured jelly reducing the need for further procedures, animals are given untreated jelly 5 days prior to surgery to acclimatise. If evidence of persistent pain beyond this time is observed then the animal will be humanely killed. Following surgery the mice will be weighed daily and monitored at least once daily for changes to normal behaviour/clinical signs (typically more frequently) as well as assessment of the surgery site for bleeding.
- All procedures will be carried out in accordance with established welfare guidelines and published scientific guidelines.

Through continual professional development, new techniques for current/new models are developed and refined through the NVS, the research community and animal technology institutions or relevant veterinary expertise.

PROJECT 51

NON-TECHNICAL SUMMARY (NTS)

Project Title	Regulation of Inflammation
Key Words	Inflammation, Oxidative stress, Innate immunity
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The aims of the project are to develop new therapies for inflammatory disease. Many human diseases such as atherosclerosis, multiple sclerosis, gout and Rheumatoid arthritis have an inflammatory component that is an important part of the pathology. Atherosclerosis, in particular, is a growing problem in Western countries so that improved treatments would contribute significantly to patient mortality and morbidity. Inflammation is closely associated with increases in oxidative stress and this work aims to develop novel therapies that target these responses as a means by which to control inflammatory responses.

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 potential benefits are that new therapeutics could be developed for the treatment of human diseases. In some cases (e.g. multiple sclerosis) there is a significant unmet medical need for new treatments as existing therapies are ineffective in many patients. The work described in this licence will also deepen our understanding of inflammatory mechanisms and how these are regulated in vivo and how they might be pharmacologically controlled.

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

Mice (adult): 3660 over 5 years Mice (neonates): 500 over 5 years Rats (adults): 60 over 5 years Rats (neonates): 500 over 5 years

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

Expected adverse effects include discomfort or pain at site(s) of injection which is expected to be mild and transient (all protocols). In some cases, (e.g. model of osteoarthritis) surgery may induce pain/discomfort which will be controlled by the use of analgesia. Some protocols will cause inflammation at specific sites (e.g. synovial joints) or systemically with moderate severity. One severe protocol will be used to examine potential therapies for inflammation of the central nervous system and may cause paralysis. All animals will be humanely killed at the end of each experiment/protocol. In the case of the severe protocol, every attempt will be made to set early humane end-points.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The molecules tested in the animal models included in this project have already been tested in cells in vitro and now need to be tested *in vivo*. This is particularly important as one of the major aims of this work is to test delivery of therapeutic molecules to sites of disease when injected systemically. This can only be tested using an *in vivo* model system.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

A statistician has been consulted so that the experiments designed have sufficient power to detect biological changes but will use the minimum numbers of animals per group. In addition, multiple parameters will be measured in each animal after death so that as much data as possible is obtained from each experiment thus avoiding the need to repeat experiments. This will not impact on the welfare of the animals while alive. Experimental bias will be reduced by use of randomisation when assigning animals to treatment groups and blinding of researchers to treatments recieved when analysing results. I am wholly committed to publish all data obtained in accordance with the NC3Rs ARRIVE Guidelines.

Refinement

Explain the choice of animals 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.

Refinement

Each of the mouse models of disease detailed in this licence are well characterised in terms of their relevance to human disease. In addition, I have carefully considered the mouse strain used in each protocol so that the severity of the procedure can be minimised as much as possible. General measures taken to minimise welfare costs to animals include ensuring that all procedures are undertaken by suitably trained personnel and by ensuring that animals are inspected at least once daily during protocols so that unnecessary suffering is avoided. Pain relief will be provided where appropriate.

PROJECT 52 NON-TECHNICAL SUMMARY (NTS)

Project Title	Fish Homeostasis in a Changing World
Key Words	Fish Physiology, Fish Behaviour, Environmental Change, Sustainable Aquaculture, Ocean Chemistry and Climate Change
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The objective of this project is to investigate the physiological and behavioural mechanisms associated with internal homeostasis (balance) and how the different physiological systems involved are integrated towards achieving appropriate maintenance of whole animal performance in fish. The specific physiological systems of interest include respiratory gas exchange, salt, water and acid-base regulation, digestion, assimilation and waste excretion. More specifically, the overall aims of this project are to investigate how endogenous and exogenous changes influence physiological homeostasis, behaviour and whole animal performance. Examples of endogenous changes to be studied include: feeding, swimming, and body size/development. Examples of exogenous changes to be studied are all aspects of water chemistry and include: temperature, dissolved gases (oxygen and carbon dioxide), acidity, salt concentrations, nitrogenous compounds (e.g. ammonia) and metals

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 project will provide an integrated understanding of physiology and behaviour of fish and how they can respond to natural and human-induced change. Understanding the fundamental mechanisms, their integration and control processes is essential to help us determine how and why such changes to the environment will affect fish populations in the wild and in aquaculture. This understanding will allow predictions about the potential impacts on whole ecosystems and the development of management strategies for the conservation of natural environments in the interest of fish populations which include fisheries relevant to human food security. Environmental managers and regulatory bodies will benefit from furthering our understanding of the impacts of future climate change and environmental pollution, and importantly leading to improve predictive capabilities. Food security, reduced environmental damage, and economic advantages to industry are also key benefits from the components of this project linked to improving the sustainability of aquaculture. Ecologists, conservation bodies, fisheries managers and global biogeochemical modellers (including our Met Office partners) will potentially be able to make use of our results in protection of aquatic ecosystems worldwide.

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

Multiple fish species will be used. In particular species relevant to aquaculture (trout, salmon, sea bass), and of ecological/economic importance to European freshwater and marine ecosystems (cod, plaice, sole, flounder, stickleback) but also fish relevant to particular environmental niches (e.g. hypersaline and hypoxia tolerant

flounder, and metal tolerant trout and stickleback). Over the 5 year project the total use of fish should be less than 4000 individuals.

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?

For the majority of fish used, only mild adverse effects are expected (at worst moderate when cumulative effects are considered), as the protocols impose conditions that are within their normal physiological limits. In the few cases where invasive procedures are used, appropriate anaesthesia, aseptic techniques and recovery procedures will be followed. Individuals will be monitored throughout anaesthesia to detect any signs of stress or suffering, and at frequent intervals following anaesthesia to ensure recovery. Any individuals perceived to be suffering undue pain, distress or lasting harm will be euthanized using a humane Schedule 1 method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

There is no alternative to using live animals, if we are to gain an understanding of the mechanistic basis of physiological responses and how they are integrated within the animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

All of the proposed methods have been tried and tested previously in numerous fish species. There is therefore a very high level of certainty that the project will yield the data that are required to address its aims. Sample sizes have been decided based on power analyses to ensure that the minimum required numbers of animals are used. The research has been designed to make use of pairwise analysis following repeat sampling of the same individuals where possible – this yields greater statistical power, and therefore enables use of smaller sample sizes of animals.

Refinement

Explain the choice of animals 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.

Refinement

For much of the research on the physiology and behaviour of fish model species have been established for which there is a good level of genotypic and phenotypic information already available (e.g. salmon, trout, sticklebacks). However, using a comparative approach with an even broader range of species to help understand the fundamental aspects of physiology and how different species vary in their ecological niche, lifestyle, and response to particular environmental conditions (e.g. salinity, pH, temperature, O_2, CO_2) is important to understand too. This allows a better appreciation of the potential for whole ecosystem impacts.

The physiological and behavioural methods used are the only available that can achieve the objectives regarding whole animal responses to endogenous and exogenous changes, both for natural and anthropogenic environmental change, and for conditions within aquaculture. These methods are also the most refined in terms of being able to detect the most subtle changes in physiological function and downstream behavioural responses, that have direct implications for understanding fitness of fish in the wild, and making predictions about how populations will be impacted, or how fish populations influence global biogeochemistry.

Also, limits on water composition would be within normal physiological limits, and physiological and behavioural tests will use the shortest period of time feasible to achieve precise data that can achieve the objectives. Monitoring of fish continuously during relevant individual steps, and at least twice daily during the whole protocol, also ensure that suffering is minimised.

PROJECT 53

NON-TECHNICAL SUMMARY (NTS)

Project Title	Modulation of joint inflammatory disease.
Key Words	Arthritis, joint model, immunology.
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

[REDACTED]focuses on Rheumatoid Arthritis (RA), a condition that affects 500.000 people in the UK. RA causes joint pain and swelling, stiffness and fatigue with 5 per cent of patients developing severe disease with extensive disability. Specifically, I aim to identify the signals triggered by structural components of joints that are responsible for changing their normal peaceful behaviour to an aggressive stage that promotes joint inflammation and sustained pain. Unfortunately, the experimental conditions used to culture cells in the laboratory do not always mimic the real arthritic joint, thereby generating misleading results. Through this project I will optimise new laboratory models, artificial joints, that take into account the physiological characteristics of a real joint.

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 understanding the biology of structural cells in the joint, we will be able to develop new therapeutics to redirect the conditions present in the arthritic joint to a normal, non-inflammatory tissue, aiming to improve the quality of life of people who suffer arthritis pain day after day. Besides, the development of artificial joints will contribute to generate more meaningful findings and ultimately, better medicines. Since our new laboratory joint model will be adapted for use with relevant human cells, a further benefit is the potential to reduce, or even replace animal research in the future, with the potential to be employed in other areas of research where similar cells play an important role in disease, such as asthma or cancer.

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

This project will require the use of mice to develop experimental models of arthritis. A maximum of 2000 animals will be employed in the work over the five-year period of the project.

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

The expected adverse effects reflect the model being employed. Some of these, for example breeding and maintenance of genetically modified animals, show no obvious indication of animals suffering any pain or stress. However, animals used to mimic human rheumatoid arthritis may suffer some pain associated with joint inflammation. Such animals are monitored continuously, for a minimum of three times per day, by researchers and qualified veterinary staff members at the licenced animal facilities. Two independent guidelines, considering clinical and general health

parameters, are used to determine when joint swelling or pain/discomfort is above the accepted levels. At this point, animals are humanely killed. All animals in all experiments are likewise humanely killed at the end of the experiment

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animal use is necessary to study structural components of joint inflammation, since there is no in vitro system that adequately mimics the complex physiologic interface that transforms these cells into aggressive pro-arthritic cells. The experimental model chosen is an accepted pharmaceutical industry standard for the investigations proposed but more importantly for this project, it shares relevant hallmarks of the human disease, making the proposed model the most appropriate biological system to optimize and generate data relating to potential therapeutic targets for extrapolation to, and validation in, the human system. Human tissue from clinical biopsies and joint surgery could be the only alternative to animal research. However, patient heterogeneity may be a disadvantage at this stage, and the consistency required to define and standardise a novel method can be only found in animal models. In addition, animal work is necessary to obtain basic understanding of early disease stages, where we do not have an alternative from clinical samples that are usually taken at late or terminal disease stages.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals employed is the minimum considered to generate enough biological material and to provide information of statistical significance to understanding human diseases and is determined in consultation with experienced university statisticians. Experimental design is made in accordance with the requirement to rationalize and reduce animal utilization whenever possible. Developing of more physiological laboratory models is a main aim for this project, as further development of this system may contribute to reduction and replacement of animal work.

Refinement

Explain the choice of animals 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.

Refinement

The mouse is the chosen species for investigating novel therapeutic targets as there are well-characterised, gold standard, industry-employed, models of human diseases that can be utilised with an unparalleled array of reagents and resources to allow elucidation of mechanism of action.

All animals will be kept in well-resourced and well-equipped modern facility, with experienced technical staff seven days/week. The Efficient Breeding of Genetically Altered Animals Assessment Tool recently published by the home office will be used to contribute to improve the efficiency and effectiveness of our genetically modified animal models. All animals are monitored continuously and any showing illness or stress, out with accepted defined levels, treated appropriately in consultation with a veterinary surgeon as required. We use non-chemical methods such as increased bedding, keeping warm and easy access to food and water to minimise animal suffering in models of rheumatoid arthritis. We have extensive experience in the protocols that are of substantial severity and have defined clear end-points and a robust monitoring system.

PROJECT 54

NON-TECHNICAL SUMMARY (NTS)

Project Title	Metabolic regulators of brain function in neuropsychiatry
Key Words	Neuronal activity, Neurotransmission, Neuropsychiatry, Metabolism
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

Neuropsychiatric conditions present a major burden for the sufferer and for the society. Depression and schizophrenia affect up to 27% of people during their lifetime. Attention deficit hyperactivity disorder (ADHD) affects up to 3.3 million children in the EU. An even larger number of individuals suffer from psychosomatic conditions, likely associated with stress and depressive symptoms, such as irritable bowel syndromes (IBS). Treatments for these conditions are often only partially effective, and their mechanism of action poorly understood, with unpleasant long-term side effects.

Peripheral factors modulated by our diet, metabolism and gastro-intestinal systems, such as metabolic/gut hormones, gut microflora, strongly interact with our central nervous system and are not sufficiently considered in relation to our understanding of the aetiology and treatment of mental and psychosomatic disorders. Indeed, such metabolic factors may have a great ability to change brain neuronal activity, central receptor sensitivity, cognition, response to stress, risk of drug abuse, and pain perception (nociception).

The project intends to explore the impact of diet and metabolism on brain neurotransmission processes involved in neuropsychiatric disorders and on their possible therapeutic implications. We will concentrate our research on three main objectives:

1) We will examine by which mechanism nutritional factors (eg gut/metabolic hormones, selective diets like those causing obesity or those affecting our gut microflora) can modulate brain neuronal activity and have an impact on neuronal processes involved in psychiatric disorders, particularly those that control emotion, cognition or addictive behaviour.

To achieve this, we will assess the effects of these metabolic factors on brain neuronal activity in vivo in terminally anaesthetised rodents, using the electrophysiological techniques which record single neuron electrical activity, and the microdialysis techniques which allow collecting brain extracellular micro-samples for neurochemical analysis.

2) We will examine how these metabolic factors can interfere with the molecular and cellular effects mediated by the main psychotropic drugs (the drugs that can have a role in treating or causing mental disorders) on neurotransmission.

Using the methods previously described, we will examine how psychotropic drugs modulate excitatory and inhibitory neurotransmission in the presence of these metabolic factors or in different metabolic situations which can alter brain neuronal activity, as may have been found in our first objective (for example following some specific diets).

3) We will determine how perception of pain is affected by metabolic and dietary factors.

By recording the electrical activity of brain pain-sensitive neurons in terminally anaesthetised animals we will examine whether such factors can modify or modulate the sensitivity of these neurons to pain stimuli, in particular the visceral pain signals that may be associated to IBS.

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 data obtained will be disseminated among the international scientific community through publications and presentations. Both scientific and clinical investigators should find these data of interest. We will also ensure that our publications will be accessible to the general public. Our data can reveal new information about the role of diet and gut microflora on brain function, particularly on cognition and nociception. They may encourage the general public to modify their diet with pre/probiotic compounds to benefit their mental health. This may have a significant direct impact on public health to manage problems as different as cognitive decline and visceral pain, which are extremely common. Cognitive decline is now a major health problem with our aging population. The study would also improve our general understanding of the regulation of brain function by metabolic regulators and may demonstrate that manipulating diet and metabolism may improve the therapeutic effectiveness of psychotropic drugs.

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

We will use small rodents, rats or mice (approximately 1600 and 350, respectively).

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 animals will be terminally anaesthetised to record brain neuronal activity. Some animals may also be treated by drugs or subject to a specific diet. These manipulations should only generate a mild degree of discomfort in most animals. For the benefit of our investigations a small number of animals will be made diabetic or hypersensitive to pain stimuli (hyperalgesic) following a specific drug treatment. These manipulations could generate moderate adverse effects which could be reduced by keeping the period of treatment as short as possible; enough to induce the pathological condition (eg hyperalgesia or diabetes), but not the long term

complications which can follow. In addition, animals will be regularly checked and can receive compensatory treatment to reduce discomfort associated with their condition (eg rehydration for diabetic animals). Animals made hyperalgesic will be subject to a pain stimuli only during terminal anaesthesia and should therefore not feel any pain. In all treatment protocols, animals are regularly monitored by trained members of staff who are supervised by our nominated veterinary surgeon (NVS). Any animals showing undue signs of distress that cannot be alleviated are immediately killed by humane method. All animals used in the study will be killed by terminal anaesthesia or other humane methods approved under the Act.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The use of animal models is crucial for the determination of long-term effects of treatments. The determination of the neuronal changes induced by psychotropic medication and metabolic factors requires the presence of neurones in an intact brain. Neurones to be examined are within a particular brain structure which is regulated by the different inputs from other brain regions. The entire central nervous system is required for such study. Whenever possible we propose to use in vitro techniques. However, in in vitro conditions cells are not in their natural environment and are dissociated from neuronal connections. It is precisely these various inputs which are of particular interest in our studies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Animal numbers will be minimised by optimising our experimental design. We use specific statistical analysis to identify the optimum least number of animals required under each protocol and this will be constantly reviewed.

We will optimise our surgical methodology and minimise the loss of animals by employing best practice at all stages following discussion and supervision with our NVS. Wherever possible, in vitro studies will be used to obtain data. Finally, whenever possible, negative results will be disseminated to avoid other groups repeating the same experiments in order to reduce animal use.

Refinement

Explain the choice of animals 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.

Refinement

Our experiments will be carried out on small rodents (rats or mice) which are the least complex species in which such studies can be successfully undertaken. Most of our experiments for in vivo measurements will predominantly be performed on deeply terminally anaesthetised animals. Only a very small number of animals will undergo recovery surgery, in anaesthetic conditions, for which the surgery time will be kept very short. Appropriate surgical procedures will be used to maximise recovery without adverse post-operative effects. Some animals may present some symptoms or discomfort associated with some treatment administration but they will be studied before they endure any long term complications. We will routinely perform appropriate regular monitoring of animals to ascertain that they are not suffering more than moderate levels of discomfort produced by the manipulation. In addition we have strict laboratory rules to reduce adverse effects (which are detrimental to the experiments) and to avoid prolonging the state of discomfort of the animals. Signs of distress in animals are usually very recognizable by our trained staff and any animals presenting them will immediately be humanely sacrificed. Our NVS regularly reviews our different protocols and inspects our research unit. He is kept informed about any issues related to the well-being of the animals and advised appropriately.

PROJECT 55

NON-TECHNICAL SUMMARY (NTS)

Project Title	Arthritis Pathology and the Impact of Therapy
Key Words	Inflammation, Arthritis, Immunotherapy
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Rheumatoid arthritis (RA) represents the most common type of inflammatory arthritis. While the clinical management of RA has improved in recent years, it is still a disease with significant unmet clinical needs. For example, there is no cure and approximately 40% of patients do not response to current frontline therapies (e.g. anti-TNF therapies) that aim to reduce joint inflammation. An improved understanding of the biological processes that drive inflammatory arthritis is needed in order to identify novel opportunities for therapeutic intervention.

RA is also a very heterogeneous disease. For example, the ‘pattern’ of joint inflammation (the cellular and molecular signatures identified though histopathology and gene expression studies) varies from patient to patient. There is a need to identify biomarkers (i.e. molecular signatures) that can inform regarding the type of joint pathology any particular patient has. Biomarkers of disease activity will help inform clinical decisions relating to the best way to manage the disease on a patient-by-patient basis.

RA is often associated with a number of other conditions (e.g. cardiovascular disease, depression) that are termed co-morbidities. We are developing novel approaches that allow us to investigate the relationship between RA and these other conditions. Promising therapies that are effective for the treatment of inflammatory arthritis will also be tested to determine whether they improve cardiovascular disease.

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

The overall aim is to improve our understanding of how the immune system causes inflammatory arthritis. This knowledge will be used to identify novel biomarkers and develop new therapies for the treatment of inflammatory arthritis. This research has the potential to benefit arthritis patients through the improved clinical management of a disease that affect a large number of people (~1% of people worldwide) and has significant socio-economic burden on the country. Outcomes of this research using animals aims to identify: (i) Novel biomarkers of disease activity in inflammatory arthritis. The clinical significance of these biomarkers will be tested in tissues from arthritis patients for development as diagnostic criteria. (ii) Novel therapeutic approaches for the treatment of patients with inflammatory arthritis. Our research outcomes are expected to inform clinical trial design in patients with inflammatory arthritis.

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

A maximum of 6500 animals will be used over 5 years. A maximum of 3000 genetically altered mice will be bred under this licence. A maximum of 3000 animals will develop inflammatory arthritis. A maximum of 500 animals will be used for pharmacokinetic studies (i.e. to test drug stability in vivo).

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?

In order to gain new insight into the biological processes that drive inflammatory arthritis, animals will be injected with biological material that causes inflammatory arthritis with clinical features very similar to human RA. This allows us to track which immune cells are important in contributing to the inflammatory response and associated damage to bone and cartilage. We will use genetically altered mice (i.e. mice lacking specific components of the immune system), so that we can determine the biological pathways that are important in driving joint pathology. This will inform us regarding which components of the immune system would make good therapeutic targets for neutralisation in the treatment of RA. Similarly, through looking at which genes are ‘switched on’ in experimental models of arthritis that match certain clinical subtypes of RA, we can identify potential diagnostic biomarkers for these different forms of the disease. Animals used in experimental procedures will be handled frequently. During these times animals will be injected (e.g. into a knee joint) under anaesthesia with biological material that causes inflammatory arthritis, and receive novel therapies (e.g. by injection or surgical implantation of a mini-pump) that have potential to improve the clinical symptoms of RA. Animals will be closely monitored throughout experiments for clinical signs of inflammatory arthritis as well as their more general wellbeing. This includes visually scoring and measuring the degree of knee joint and/or paw swelling with fine callipers. A small amount of blood or urine may be collected from some mice for analysis. Through good handling techniques, distress caused to the animal from being restrained will be minimised in terms of time and discomfort (an animal will typically be restrained for less than 30 seconds at any one time). Should the development of arthritis limit mobility, feeding or drinking, or the clinical signs reach a pre-defined maximum score, animals will be killed by a Schedule 1 method. Some animals with arthritis may undergo behavioural analysis or imaging analysis. This is an opportunity to examine the exploratory behaviour of animals in their environment and, for example, can provide new information relating to the degree of anxiety and pain associated with arthritis. This will help refine our future use of these experimental models. At the end of the experiments, mice will be killed by a Schedule 1 method and tissues (e.g. joint tissue and peripheral organs where we can monitor immune responses) recovered for cellular and molecular analysis. Based on our experience of working on models of inflammatory arthritis since 2006 the animals used in this project not exceed the “moderate” severity limit.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

During the development of inflammatory arthritis, a complex network of communication and movement occurs between different types of immune cells, and stromal cells that form tissues. With this in mind, an animal model is necessary as there is no other way to recreate these *in vivo* conditions in *in vitro* cell culture systems.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Experiments will be designed based on previous findings, which will inform the minimum number of animals needed to achieve statistically significant findings. When this information is not available (e.g. the first time a new therapy is trailed) small ‘pilot’ experiments will be performed to ensure that the number of animals used is kept to a minimum. Where possible, this project will use longitudinal analysis (e.g. *in vivo* imaging) in individual animals thereby significantly reducing the number of animals needed to evaluate arthritis over time.

Refinement

Explain the choice of animals 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.

Refinement

Mice and Rats provide an excellent model in which to study the immune system. They are well characterised immunologically, and their immune systems closely resemble those of humans. For mice in particular, the availability of genetically altered strains allow us to investigate specific immune functions in relation to arthritis development.

We have significant experience in the induction of inflammatory arthritis in animals. Arthritis will be induced through injection of arthritis-inducing proteins, which involves only mild, transient discomfort to the animals. Development of arthritis is monitored closely and does not impede the animals’ ability to feed, drink and explore their environment. Where appropriate, analgesics will be added to drinking water. We

have introduced soft bedding material that reduces the chances of limbs getting tangled and arthritic animals are handled on soft surfaces (e.g. VetBed). Formal assessment of arthritis development will be performed frequently. In the unlikely event that animals reach certain pre-defined clinical scores (e.g. joint swelling) or display unexpected adverse responses they will be killed by Schedule 1 method to avoid pain / discomfort.

PROJECT 56

NON-TECHNICAL SUMMARY (NTS)

Project Title	Investigating new radiotherapy and drug treatments for primary and secondary brain tumours, lung cancer and mesothelioma
Key Words	Radiotherapy, resistance, glioblastoma, mesothelioma
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall aim of this project is to develop relevant mouse models of brain and lung cancer, and use these models to identify novel therapies that can increase the effectiveness of conventional cancer treatments (i.e. chemo and radiotherapy). Both glioblastoma (brain cancer) and mesothelioma (lung cancer) have a dismal prognosis with treatment resistance playing a key role in tumour recurrence and progression. The work out lined in this project will aim to investigate the biological mechanisms underlying treatment resistance with a view to develop more effective treatment strategies. Our plan to achieve this (1) to test what tumour cell lines grow tumours in mice (this will involve intracranial surgery for the glioblastoma cell lines), (2) characterise tumour biology using MRI scans and histology to identify pathological features and expression of drug targets in the tissue, (3) use the best characterised tumour models to test novel radiotherapy-drug combinations (this will involve administration of drugs to mice, irradiation of mice and MRI scanning), (4) investigate the adverse effects of radiotherapy on normal tissues using behavioural testing, MRI and histology to determine changes in tissue physiology and structure.

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

• This project will increase our understanding of glioblastoma and mesothelioma biology, which could identify mechanisms of treatment resistance and how to overcome these. • Clinically relevant radiotherapy and drug combination treatments will be tested in the models being developed. [REDACTED] Therefore, the preclinical work that we propose to run in parallel will be completely relevant with translational promise. • Ultimately our scientific findings with mice could make an impact on cancer sufferers by supporting these trials.

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

• We predict to use approximately 3600 mice over 5 years for studies involving tumour bearing mice, whereas normal tissue toxicity studies are predicted to use 1500 mice over 5 years.

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

Injection of tumour cells in to the mouse brain in order to grow brain tumours, or creation of a window on the mouse skull to look at the behaviour of tumour cells in the brain (under a microscope) requires cranial surgery. The mice are under anaesthesia throughout the surgery and given pre and post-operative pain relief. After surgery mice are kept warm using a heated cage rack and returned to their cage once fully awake and mobile. Mice are regularly health checked by competently trained staff using monitoring charts that list symptoms that could be experienced by the mice under procedure. The most common symptoms expected with regards to intracranial tumour growth are weight loss, subdued behaviour and in some cases seizures. Mice displaying such symptoms are humanely killed. Mice treated with radiation may experience anaemia, diarrhoea, paralysis or abnormal behaviour patterns. Where possible, we will use non-invasive imaging techniques as a method of monitoring tumour growth and response to therapy. All imaging techniques require anaesthesia and may be performed on more than one occasion. At the end of each experiment mice are humanely killed and the tissues harvest for further testing. The described procedures have moderate levels of severity.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The failure of cell culture models to recapitulate key features of brain tissue is a likely factor explaining why most new treatments developed in the laboratory have failed to provide benefits for patients with glioblastoma. To investigate this further, [REDACTED]. However, despite the advancements in our cell culture models, these assays cannot fully model the complexities of cancer development in the living organism. It has been well-documented that both immune system and tissue components play an important role in disease progression and these factors cannot be fully recapitulated in the test-tube, thus the requirement of animal studies remains.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Non-invasive imaging methods will be used to monitor tumour growth and responses to therapy. This will avoid unnecessary killing of animals at different time points, and allow for longitudinal studies that are more statistically powerful. Testing of cells in conventional and novel culture models on plastic will be undertaken to identify promising agents prior to testing in mice. Pilot studies with fewer numbers of mice

will always be performed when using new cell lines and therapies. Data from pilot studies will be used towards power calculations of sample size for larger quantitative experiments.

Refinement

Explain the choice of animals 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.

Refinement

This licence uses immunocompromised mice to grow tumours of human origin with procedures that are no greater than moderate severity. It is essential that we use immunocompromised mice in order for non-mouse cell lines to grow tumours successfully without rejection by the host. Wild-type mice (which have a full, uncompromised immune system) may also be used in studies were the immune response is thought to play a key role. Pilot studies will be performed when using new tumour cell lines to determine the take rate and for characterisation of tumours. This will determine if a full-scale experiment is merited and will help answer scientific questions efficiently.

To minimise suffering, all mice on procedure will be frequently monitored and humanely killed when exhibiting signs of altered health status and/or tumour burden. All users will be fully trained in monitoring tumour development for each model and will be signed as competent prior to initiating their own studies. Dedicated monitoring sheets list clinical signs and give classifications of mild, moderate or severe to clarify to users when mice should be killed. Our animal unit is proactive in environmental enrichment and provides fun tunnels and nesting materials in cages.

All surgeries will be performed in a dedicated surgical suite and always using aseptic techniques. Pre and post-operative analgesia, and anti-microbial therapy (where required) will be administered routinely to animals under-going surgery as advised by the vet and in line with current guidelines.

PROJECT 57

NON-TECHNICAL SUMMARY (NTS)

Project Title	Nucleic Acid Sensing by Innate Immune Receptors
Key Words	immune response, vaccination, cancer
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Viruses are infectious agents that cause a variety of diseases, ranging from a common cold to AIDS. The immune system can eliminate viruses, and we are trying to understand how the immune response is kick-started upon infection.

The first step is that the cells in our body recognise the presence of a virus. We know that cells have specialized proteins called receptors that detect viruses. However, how these antennas sense viruses is largely unknown. By investigating the mechanisms of detection we hope to understand how the immune response is initiated during virus infection.

One of the hallmarks of this anti-viral immune response is the production of a group of molecules called interferons. The name stems from the property of interferons to interfere and block the replication of viruses. Interferons achieve this by instructing cells to switch on their antiviral defences. Interestingly, interferons are not only essential as central players in antiviral immune responses. They are also produced during vaccination and are necessary for the development of protective immunity. Moreover, interferons are involved in cancer and may help our immune system to fight malignant cells. These new areas of research hold great promise for the development of new vaccines and novel cancer treatments. We want to obtain a better understanding of the underlying biology, which will be required for the development of new medicines.

Despite all these beneficial functions of the immune system, it is a double-edged sword and can cause problems, too. Patients suffering from autoimmunity are not infected with viruses or other pathogens, but their cells activate a long-lasting immune response that damages the body. Our hypothesis is that the immune antennas are not tuned to the right signal. We hope to reveal why the immune system is tricked into this false alarm.

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

The primary potential benefit of this work relates to new knowledge in the area of immunology. Our findings may allow us to develop new ways of boosting immune responses to more effectively eliminate dangerous viruses. Being able to better activate immune responses may also advance vaccination strategies and instruct ways to develop new treatments for cancer. In addition, we envisage inhibition of immune sensors as a treatment in autoimmunity, and our work may provide insights towards such approaches.

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

We will use mice as an animal model, including genetically modified mice that lack specific immune receptors or related molecules (up to ~24,000 animals over 5 years).

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

We will breed genetically modified mice. In most cases, the genetic modification will not cause any adverse effects. In some instances, genetic modification may lead to the development of autoimmunity. Manifestations of this include behavioural changes and weight loss. These will be carefully monitored, with clearly defined thresholds such as 15% weight loss, and animals will be killed immediately if these thresholds are reached. The majority of animals will be humanely killed without undergoing procedures and tissue will be used for experimentation. In addition, some animals will be used in models of virus infection, autoimmunity, vaccination and cancer. These models involve administration of viruses, substances or cancer cells. We will use injection, inhalation and the drinking water to administer these agents. Most animals will not suffer at all or will experience only mild and transient adverse effects such as tenderness around the injection site, which typically self-resolves within 24 hours. In the infection, autoimmunity and vaccination models, a small number of mice (less than 10%) may suffer adverse effects that last longer, and this will include weight loss and behavioural changes. In our cancer model, tumour development will occur in all animals. We will regularly monitor animals undergoing procedures and will record and measure adverse effects. Weight loss will not exceed 15% and tumour diameter will not exceed 1.2cm. Animals will be humanely killed immediately if these thresholds are reached, or before if scientifically possible.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

To answer our scientific questions, our project integrates multiple scientific approaches. This includes biochemistry and molecular biology in the test tube wherever possible to dissect individual aspects of immune recognition. For example, this involves using cells isolated from animals humanely killed by Schedule 1 methods and using existing cell lines. However, we also need to use an animal model because the immune response is a complex process involving many different types of cells and molecular mediators. There is no feasible alternative that would

entirely replace the use of a living animal. Where work not involving protected animals is insufficient to achieve our research goals, we will use mice as an animal model, including genetically modified mice that lack specific immune receptors or related molecules.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use statistical models to determine the minimum number of required animals. We will also design experiments in such a way that many data points can be collected from the same animal. We will use male and female animals, which reduces the number of surplus animals. We will use a breeding strategy - managed by staff trained specifically in maintenance and breeding of mouse colonies - that keeps the number of mice to a minimum. Unwanted genetic changes will be prevented by regular crosses to a reference mouse strain. Finally, experiments will be blinded as much as possible to avoid bias. Taken together, these measures will allow us to obtain robust and reproducible data from a minimum number of mice.

Refinement

Explain the choice of animals 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.

Refinement

We chose mice given that their immune system is sufficiently similar to the one in humans to draw conclusions that are likely to equally apply to man. Once the scientific objective of any procedure has been attained the animal will be disposed of humanely. Specific humane endpoints will be applied. We have chosen those models of virus infection, vaccination, cancer and autoimmunity that are most refined and cause the least possible harm. We will are fully monitor all animals involved in the study and continuously seek to identify new methods for refinement.

Specifically, our virus infection models interrogate the early stages of infection. At these time points, the innate immune system becomes activated and we will study this process. However, at these time points, virus replication has not yet resulted in tissue damage that causes profound disease. Animals will be culled before they reach this later stage. Similarly, in our autoimmunity studies, we are using slowly developing disease models instead of acute onset, severe models. This allows us again to focus on early stage of innate immune activation and to stop procedures before animals become more strongly affected.

Other examples of refinement applied in our work are: (a) Freund's adjuvant will not be used. This is a component of vaccine formulations that has been used in the past and caused adverse effects such ulcerations. We will use other adjuvants that do not cause adverse effects. (b) We will use tumour models that are easy to monitor and do not form secondary tumours (metastasis). (c) Footpad injections will not be used and will be replace with a refined model (hock injection) that is much less painful but achieves similar scientific aims.

PROJECT 58

NON-TECHNICAL SUMMARY (NTS)

Project Title	Safety and efficacy assessment for fish medicines
Key Words	vaccine, safety, pharmaceutical, fish, efficacy
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

This Project Licence covers the *in vivo* testing required to demonstrate that medicines destined to be used to treat farmed fish are effective and safe to the fish themselves and the consumer.

Severe diseases caused by infectious disease agents (bacteria, viruses and parasites) cause significant losses of farmed fish. Such diseases cause significant welfare problems for the animals concerned, a waste of resource inputs, and economic losses, constraining the sustainable development of this important industry. Veterinary medicines and vaccines are needed to treat or prevent these diseases, but their effectiveness and safety to the animal, to the consumer and to the environment must be established first. Whilst much of this can be done by *in vitro* methods not using animals, it is still necessary to confirm that a candidate treatment truly protects or treats the target animal species from the disease concerned and is safe for such use. The final stage of consumer safety, to protect consumers from unacceptable veterinary residues and to determine the minimum length of time between last treatment and slaughter, must be tested in the animal itself, because currently available artificial systems do not offer sufficient guarantee of consumer safety.

The fish species used under this project will be the farmed fish that the treatments are designed to protect, or treat, thus ensuring the results are fully transferrable to potential use in the field.

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 results of the tests carried out under this Project Licence will help improve / maintain the availability of aquatic medicines, vaccines and feed additives, and thus markedly assist in maintaining the welfare of the more than two billion fish on fish farms in Europe. In addition, they will also support efforts to protect consumer safety by provision of incurred residue materials for testing laboratories looking to developing analytical methods to detect the presence of legally and illegally used medicines in fish meat destined for human consumption.

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

The types of animals to be used will be fish species, principally Atlantic salmon and rainbow trout. Other species will also be used, such as sea- bass, carp, tilapia, wrasse, lumpfish and other cyprinids and, turbot and dab. Numbers are difficult to predict as these will depend on the numbers of products to be tested over project,

types of testing required and, in particular, the size of fish to be used. An estimated range is approximately 7,000-40,000 over the 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?

For most of the safety tests, no adverse effects are likely. For the efficacy testing, this will involve exposing fish to harmful pathogens, which will result in severe disease symptoms, including mortality. However, care will be taken to identify humane endpoints where possible to reduce suffering to a minimum

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

For much the work done under this Project License to test candidate veterinary medicines, EU and UK regulations typically require use of live animals, since the objective is to determine the safety and efficacy of the product in the whole animal. The whole animal in most cases needs to be the intended target species for the veterinary medicine.

This also applies to studies where we will look to expose fish to illegal medicines in support of testing programmes that help confirm that fish sold to the public is free of illegal contaminants. We need to be sure that the laboratories running the testing have access to samples of fish tissues that have been dosed ('incurred') with these drugs in a way that fully mimics the way they would accumulate in nature. At this time, these studies must still be carried out on the whole animal

However, advice and guidance from advisory bodies, along with published information, will be continually monitored throughout the duration of this licence and where non-animal testing is accepted by regulators these will be adopted in keeping with the principles of replacement.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The minimum numbers of animals to be used are as defined by the relevant regulations, or, where this is not mandated, by consultation with our statistical services group in each case, to ensure the minimum statistically valid number of fish,

consistent with the need to provide robust data, are used. This also includes a careful check of the published literature to ensure the work planned does not duplicate other work that has already been undertaken. [REDACTED]staff also regularly discuss planned tests with the regulators themselves (e.g. the UK's Veterinary Medicines Directorate) to ensure the most appropriate studies are undertaken, consistent with the principles of the 3Rs. This all helps to avoid unnecessary use of fish.

For each and every experiment involving live animals (fish), we then write a Study Plan which includes:

- A statement of the objective(s)
- A description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material
- How the 3Rs are addressed.

The protocols are then reviewed by our local Animal Welfare and Ethical Review Body (AWERB) to ensure the studies are ethically justifiable and adhere to the principles of the 3Rs. No study can take place until it has obtained clearance from both the statistical services group and the AWERB.

Refinement

Explain the choice of animals 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.

Refinement

The target species used will typically be the farmed species to eventually be treated with the products, this may also be as mandated under relevant legislation. These include salmonids (Atlantic salmon and rainbow trout), sea-bass, tilapia and carp. Although these will form the majority (certainly in terms of numbers) of experimental fish, the list cannot be exhaustive; other species may become important during the life of this licence (e.g. lumpfish).

Stocking density and population size will be considered to ensure expression of normal feeding and social behaviours, and to minimise anti-social (aggressive) behaviour.

The fish themselves will typically be purchased from normal fish farm stock (or reared from eggs in house), usually from a site with an established disease history as disease-free fish in compliance with relevant regulations.

Suffering will be minimal in the protocols assessing the safety of medicines, as most of the procedures listed will be no greater than mild. Where preliminary tests with

candidate products are required, and there is not good toxicology data available for the target species, it is possible that occasional moderate reactions will be observed. A literature review will be undertaken to determine test doses that are unlikely to cause any adverse reaction. In practice, it is unlikely that adverse effects that would even be considered moderate will be observed in these protocols.

Other than that, methods used under those protocols are identical to those that will be used by veterinary practitioners when applying antibiotic therapy (or other pharmaceutical treatment) to control disease. No adverse effects are expected and if any occur the trial will be terminated immediately.

Where the fish are dosed with an illegally used veterinary medicine or environmental contaminant, a literature review and consultation with aquaculture health and other experts will always be undertaken first to establish a likely safe dose believed representative of practice. Where such published data or information are scarce, a pre-test will be carried out with a small number of fish (10 or fewer) applying a dose believed representative of practice. This is final confirmation that the proposed dose is safe before exposing much larger numbers of fish to produce incurred residue material.

Severe protocols

Challenging fish with pathogens or toxic substances, as is undertaken for many of the vaccine and medicine efficacy tests, often results in the death of the challenged fish. In some cases, where European and UK law require it, there will be no option but to use death as an endpoint (e.g. European Pharmacopoeia monographs for fish vaccines). It is otherwise proposed to use infection or moribundity as alternative end-points to mortality for other studies performed under these protocols. On occasion, particularly for vaccine tests, it will also be possible to monitor the host response of treated fish by serological or other methods. Where this is scientifically justified, this alternative approach will be followed. In those cases, treated (e.g. vaccinated) fish will be sampled at intervals and blood samples examined to follow the host response to the vaccine. Efforts will be made to influence EU and UK regulators to mandate the use of alternative criteria to death as an end-point, where published guidance specifies the use of death.

PROJECT 59

NON-TECHNICAL SUMMARY (NTS)

Project Title	Stem cell regulation in self-renewing tissues
Key Words	stem cells, intestine, regeneration, cancer, mouse
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Adult epithelial tissues, such as the gut, are maintained and repaired by the action of resident stem cells, which are uncommitted cells capable of dividing into new unspecialized stem cells while also producing specialized cells. Accurate control of stem cell behaviour is essential to maintain proper tissue size and shape. Uncontrolled stem cell division can lead to diseases including cancer.

Our objectives are:

1. Learn about the mechanisms that maintain normal self-renewal rate of tissues.
2. Understand how normal self-renewal mechanisms are co-opted or affected during cancer
3. Understand the processes involved in the repair of tissues in response to damage.

For all the objectives described above we will focus on the role of stem cells in those processes.

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

We hope to achieve an understanding of universal basic biological processes, which are part of human health and disease and which will ultimately contribute to the development of useful therapies directed to the treatment of cancer and tissue repair.

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

Mice. Between 6,000 and 7,000 animals over 5 years with less than 50% of them undergoing any given procedures.

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

Animals will be bred and induced to generate epithelial tumours or tissue regeneration. From the Animals used for regeneration studies or just after breeding are unlikely to suffer any adverse effects. These 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. Animal bred for tumour development will be predisposed to cancer and will be monitored carefully for clinical symptoms. Symptoms include paling of feet, anaemia, weight loss, swelling of the abdomen and development of visible or palpable

tumours. Carefully trained staff will monitor mice with tumours and if the tumours interfere with normal behaviour, become larger than allowed by guidelines, or have any consequence greater than allowed by guidelines, mice will be humanely killed and the tissues will be analysed. Tumour cells will be grown in the laboratory. At the end of any study, all animals will be euthanized.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Our laboratory uses adult fruit fly intestine as a primary model system. We use this genetically tractable organism as our intitial and main platform to tests our hypothesis and assess the function of multiple genes in the contest of a living animal (in vivo). As an alternative to the use of mouse models, translation of our fly work into mammalian models will include the use of pre-establised mouse tissue organoids and human tissue samples obtained from colleagues before doing in vivo mouse work.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

At the moment of using live mouse models we will take a very focused approach by modifying just the candidate genes, which have previously been identified to have a clear effect in the intestine of our fly models. This will greatly reduced the number of animals used. Additionally, using already available mouse tissues previously extracted by other researches within our institution and tissue samples from the biobanks will provide the means to translate results from our fly work without having to use addional animals. We therefore will keep our use of mouse models to the minimun and only to validate the potential significance of our most solid fly data into human health and dissease.

Refinement

Explain the choice of animals 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.

Refinement

We use state-of-the art genetic models to ensure that genes are removed or activated in the correct organ/tissues to reduce side effects. When using whole mutant animals it will be done under the premises that this does not affect overall animal health. Animals will receive anaesthetic and/or analgesic treatments where appropriate. All animals will be monitored regularly for signs of normal behaviour and will be humanely killed if they exhibit moderate adverse signs.

PROJECT 60

NON-TECHNICAL SUMMARY (NTS)

Project Title	Kainate receptor-dependent plasticity and its role in brain development
Key Words	Autism, Kainate receptors, Synaptic plasticity, Glutamate receptors
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or

	improvement of vocational skills;
No	(g) forensic inquiries.

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

Nerve cells communicate and transmit information across structures called synapses. The sending nerve cell (presynaptic) relays the information by releasing chemical transmitters. The receiving cell (postsynaptic) detects that signal by specialized receptor proteins present at the synapses and modify the way neurones are connected. In the adult, synaptic connections can change depending on how they are used: busy synapses can become stronger or they can become weaker and even completely disappear when poorly used. These processes, called long-term potentiation (LTP) and long-term depression (LTD) of synaptic plasticity sound simple but, in fact, they require a highly regulated and coordinated series of events that are the cellular basis for memory formation and learning processes.

Sometimes, an improperly orchestrated LTP or LTD activity can occur, manifesting as cognitive deficits. This is the case for many neurological disorders such as dementia, Alzheimer's Disease or intellectual disability. In addition, this plastic remodelling of the brain influences the correct formation of neuronal networks during the childhood. In this stage, what we see, hear, touch, taste and learn, will shape specific circuits in a manner that they are reflecting the experience incoming form the external world. The main target of the project is a protein called kainate receptor, which is present at the synapses.

My goal is to explore a new and unusual way in which kainate receptor activity can strengthen or weaken the synaptic connections, thus affecting the power of our brains to learn and memorize new things. In addition, kainate receptors are present in very high levels at young synapses, when the external experience is shaping them, and are reduced as the development progresses and the adult patterns of neuronal circuits and connectivity are established.

How kainate receptors modulate other receptors in the synapse will be studied first, and then the synapse capacity of being potentiated or depressed and eliminated to form a normal network of connections will be tested. This is important because when it goes wrong, it is believed to cause disorders such as autism, schizophrenia and intellectual disability. If so, the way to impede and prevent such abnormality will be sought, which would indicate a new way in fight against these neurological pathologies and its devastating consequences.

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)?

Though knowledge of causes of autism has advanced, the role of many factors operating normally in the brain development is still unclear. Existing data indicate that kainate receptors play a role in early processes that will determine the fate of the final brain organisation. Understanding how kainate receptors contribute to establishing the healthy interconnections in the brain will help us try to find the way to prevent the consequences of inappropriate connectivity. This will help us devise a new strategy in the fight against autism and its devastating consequences.

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

Mice, 2200.

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

The animals will be genetically modified, but this is not expected to cause any adverse effect by itself. Their tissues will be used after death for the imaging and electrophysiological studies. Anaesthesia and analgesia will be used as necessary and any animal experiencing an unexpected adverse effect will be treated as advised by the NVS or will be killed humanely.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Currently, there is no satisfactory alternative model for investigation of the mechanisms of synaptic changes in autism models that does not require the use of brain tissue acutely removed from animals.

The project is intended to result in development of the new transgenic mouse strains engineered to evaluate the role of kainate receptors in development of brain connectivity. Therefore, this requires maintaining viable breeding colonies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Using the preliminary data, we have used validated statistical procedures to calculate the minimal number of animals necessary to produce meaningful data, without compromising the scientific validity of the study. In addition, the tissues will

be shared with other groups to ensure that neuronal and non-neuronal tissue from the animals is used to the fullest extent possible.

Refinement

Explain the choice of animals 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.

Refinement

We chose mice as the species widely used in transgenic animal design, while also simultaneously validated as the species of choice by current scientific literature. Further, there is a wealth of correlative studies between mouse and human which indicate that the results gained by the animal use are translatable.

All of the procedures I propose: a) are validated in current scientific literature b) will be performed according to the relevant legislature and c) will be performed by trained staff.

Mice will be monitored on a daily basis and for any animal that shows signs of adverse or unexpected responses, depending on the severity, either the advice will be sought from the local NACWO and/or NVS or the mouse will be culled immediately to limit any additional discomfort.

PROJECT 61

NON-TECHNICAL SUMMARY (NTS)

Project Title	Novel and advanced therapies for heart failure
Key Words	cardiac, gene therapy, cell therapy, heart failure, myocardial infarction
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

Myocardial damage (such as heart attacks, genetic defects or damage from chemotherapy) not only reduces the power of the heart to contract but leads to further ongoing damage as the body overstimulates the remaining heart muscle. This leads to heart failure, a condition with severe symptoms and a prognosis as poor as some of the worst cancers. Patients die from failure of the heart to beat strongly enough or, in about half the cases, to sudden disturbances of rhythm (arrhythmias). Drugs in use presently for heart failure concentrate on preventing further damage but do not reverse the condition. Advanced therapies such as new kinds of drugs, or gene and cell therapy aim to produce greater benefits and ultimately restore full function to the heart.

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)?

For gene or drug therapy, the aim is to restore full function to the undamaged muscle in a safe way, and to reduce arrhythmia. Clinical trials are already in progress as a result of our work, and the aim for the animal studies now is to improve the therapy as a result of what we learn from these trials. For cell therapy, the more ambitious aim is to give new muscle back to the heart using stem cells. For this, tissue engineering strategies using materials are likely to be the best way to deliver the stem cells

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

Over 5 years we will use an absolute maximum of 8200 mice; 4800 rats; 410 guinea-pigs and 1250 rabbits. The total is likely to be considerably less than this as we have listed a number of alternative protocols

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?

For the operations to induce heart failure (aortic banding and myocardial infarction), or to introduce gene or cells, there will be an open chest procedure. This has the potential to have similar pain levels to a human heart operation, and will be performed in the same way with full anaesthetic, and painkillers given during and after the operation. Mimicking the damage caused by chemotherapy is done by tail vein injection under anaesthetic: however the drugs can be irritant so pilot studies have been done to reduce these effects as far as possible. Animals will be followed for weeks to months to determine the development of heart failure and the effects of drug, gene or cell therapy to reduce it: they will be imaged serially during this time.

Around 50% of the animals may undergo sudden cardiac death due to arrhythmia when heart failure occurs. Loss of consciousness is thought to be rapid, so that this death is relatively fast and painless. Others can develop symptoms of heart failure which are breathlessness, fatigue, water retention causing some paw swelling and blue extremities due to poor oxygen supply to tissues. Usually, we can predict when the heart is starting to fail from the imaging studies, and so few of the animals reach the point where symptoms are seen. At the end they will be killed by approved humane methods and hearts taken for further studies where live tissue is investigated using state-of-the-art imaging.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Heart failure is a condition where the responses of the body drive forward the disease process: this cannot be mimicked in an isolated heart outside the body. Similarly, the effect of gene or cell therapy to improve the function and prevent further damage must be done in a live animal. However, we gain a great deal of further information by using tissue and cells taken from the animal and used outside the body. We also perform similar experiments using human tissue from surgical specimens when we can, but these are available only 5-10 times per year and never include healthy tissue. We have been developing, as a replacement, heart muscle cells derived from human stem cells. Many of our experiments are now done on these cells, which we can also reconstitute into a functional muscle strip. We are using patient material as a source to derive these stem cells in order to investigate the effect of genetic modification on heart muscle cell function.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

For the protocols listed, funding has been obtained or will be applied for before going forward. Funders require power calculations to match animal numbers to the outcome, and these are peer reviewed by expert scientists. The Boards of many funders now include statistical advisors to assess these power calculations. Randomisation and blinding to reduce bias and improve reproducibility of models will be used at appropriate states in the experiments. We commit to publication in line with the NC3Rs ARRIVE guidelines

Refinement

Explain the choice of animals 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.

Refinement

It terms of the objectives, there are different specific advantages of the various species. Mouse gives the power of the transgenic models for investigating human cardiac mutations. Rat produces a reproducible human-like myocardial infarction and develops heart failure gradually over a controllable period. Guinea-pig and rabbit both have myocardial electrical characteristics closer to human heart, which is important for the integration of human stem cells. Rabbit heart thickness is more suitable for testing larger material/cell constructs intended for man. We wish to develop this as an alternative to pig or primate as a pre-clinical model.

In general we will match the monitoring frequency to maximise the chance of adverse effects being captured. In all cases where adverse effects are noted, advice will be sought from the named animal welfare officer and/or named veterinary surgeon and humane euthanasia will rapidly be used for any animals not responding to treatment given. All surgery will be done under general anaesthesia: pre and post-operatively the pain will be controlled with analgesics as for human. For the chemotherapy protocol, tail vein injection will be done under light anaesthesia and analgesics use afterwards. Thorough flushing and washing will remove the irritant drug from the site of injection. Heart failure as a result of the various strategies is seen in symptoms such as fatigue, paw swelling from water retention or breathlessness. Imaging of the heart will be done to catch the animal just before these symptoms emerge, but as soon as they are seen this is considered a humane end point. Some animals will die suddenly from disturbances of heart rhythm, as with heart failure patients. The human experience from resuscitated patients (as well as our own observations of mice) suggests that this occurs in minutes with immediate loss of consciousness, so the distress has a limited duration.

PROJECT 62

NON-TECHNICAL SUMMARY (NTS)

Project Title	Study of antimicrobial resistant bacteria in mice
Key Words	antimicrobial resistance, bacteria, mice, transmission
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No	(g) forensic inquiries.

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

The overall aim of the project is to ‘map’ the spread of drug-resistant bacteria in the intestine. Current approaches to study the spread of resistance are limited and favoured towards detecting organisms that are easy to grow in the laboratory. [REDACTED] We will use this system to study how drug-resistant bacteria spread in the intestine during treatment of the host with antibiotics.

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)?

Generating a ‘map’ that describes which organisms are drug-resistant in the intestine will help us identify key reservoirs. This knowledge could allow us to apply more targeted interventions to reduce their populations. Within the context of a host, this could reduce the number of drug-resistant organisms released into the environment and reduce the risk that other members of the population could acquire the drug-resistant bacteria.This information will be of use to both human and animal populations.

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

This project uses laboratory mice (Mus musculus) and we plan to use approx. 1750 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 levels of severity? What will happen to the animals at the end?

The animals will be given orally different strains of resistant bacteria, and some mice may be treated with different types of antibiotics. Depending on the type of bacteria, the mice may develop mild disease (manifest as transient weight loss over a few days) or no disease at all. Antibiotics may cause transient intestinal upset but this is unlikely to result in disease. All mice will be euthanised at the end of the experiments (based on defined experimental or humane endpoints).

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Only living animals can reproduce what happens when bacteria are ingested and subsequently released from the body in faeces – the natural route of transmission of

food- or water-borne organisms. This is because the biology of the bacteria is altered during host passage and this can affect the ability of the organisms to persist and survive outside of the host. Non-animal alternatives will be used where possible but most alternative approaches are limited as they do not allow the full complexity of the intestine (with its natural microorganisms and chemical/physical gradients) to be reproduced. Additionally, non-animal alternative do not allow host-to-host spread to be studied.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used in this study has been estimated based on what would be required to obtain publishable data with the commensurate statistical significance. This was calculated after reviewing similar published data and seeking the advice of experts in statistics and experimental design. Following the initial experiments, estimates for the number of animals per group will be reviewed and adjusted as necessary.

Refinement

Explain the choice of animals 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.

Refinement

Mice are used in this project. Mice are easily-manipulated and harbour a well-characterised microbial population in their intestines, the diversity and complexity of which is similar to humans. Other animal species contain less well defined microbial populations (rats), are higher sentient and less easy to manipulate (rats, pigs) or have intestinal systems that are fundamentally different from humans (Drosophilia, zebrafish). Harms to the mice are minimised as the bacteria and mice breed we use have been chosen to produce high levels of intestinal colonisation with minimal disease. Timely monitoring and careful observation of the mice will ensure that any harms are kept to a minimum and when they occur, dealt with promptly.

PROJECT 63

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Cell proliferation, repair and integrity in the gut
Key Words	Nutriceuticals, Dietary treatment, Gastrointestinal damage, Gastrointestinal repair
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The experiments performed in this project will investigate how the gastrointestinal tract adapts and repairs itself after injury caused by various means. It is important to find out how this works as it could lead to therapies for gastrointestinal damage caused by ulcers, cancer treatments or operations.

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 gastrointestinal tract is constantly being damaged by food, digestive enzymes, chemicals (such as aspirin and alcohol) and acid. It is important that the lining of the gastrointestinal tract remains intact to stop any of these entering the rest of the body. Therefore, the cells in the lining of the gastrointestinal tract need to constantly be renewed by proliferation, so that the defence, barrier function but also the ability to absorb food remains working. When the lining of the gut gets damaged the first thing that happens is that cells from the edge of the injury start moving across the damaged area (restitution) to protect the area underneath. This happens within hours of the damage occurring after that new cells are made (proliferation) to repair the damaged ones, this happens within 24 hours of the damage occurring. In addition to every day damage the gastrointestinal tract can also be injured by things such as chemotherapy, irradiation, non steroidal anti-inflammatory drugs, strenuous exercise, mechanical injury or injury caused by illness. In this project we intend to investigate if various factors and naturally occurring (health food) products can help reduce or repair the gastrointestinal injury caused. Possibly leading to new human therapies.

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

We plan to use rats and mice, to achieve our research objectives. Over the next 5 years, we will use on average less than 450 animals per year if we don't produce genetically modified animals. If we do use genetically modified animals this will require the use of an additional 200 animals per year. It is anticipated that usage will be much lower than this.

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

We expect the damage induced to be mild to moderate and in exceptional circumstances severe. However, we have used these models for many years and have learned how to minimise the chances of any unnecessary suffering or harm. Animals should show no overt signs of pain; however, we have strict criteria in place to assess pain and suffering, and if these criteria are met, he or she will removed from the study and killed. All animals will be killed at the end and their tissues taken for further study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Whenever possible experiments using cell culture (human cell lines) are being used to understand this area. However, the experiments described in this project are essential to allow us to understand how a complex system is regulated. The gastrointestinal tract is a complex organ that performs many tasks that interact with each other. This cannot yet be adequately replicated by using cell culture or other non-animals approaches.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

[REDACTED]All studies are carefully planned and our usage has reduced progressively over each project license period. We will use data from our previous published studies to estimate the minimum number of animals needed to show a true effect from a treatment whilst at the same time maintaining sufficient numbers for the experiment to be meaningful. Advice from biomedical statisticians has been sought and will continue to be sought when appropriate. We will continue to ensure that any

tissues generated from our work are archived and stored appropriately, therefore preventing unnecessary repetition of experiments.

Refinement

Explain the choice of animals 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.

Refinement

We will use rats and mice because they are the species whose gastrointestinal system is best understood, and they can be used to give insights into the mechanisms underlying inflammation and repair in the human intestine. I work closely with the animal care staff to ensure that the animals do not suffer or experience pain. I treat the mice with great respect, as living beings. Results from each set of experiments will be reflected upon before embarking on a new series to ensure that the minimum numbers of animals are used and that the protocols being followed are most appropriate to obtain useful data.

PROJECT 64

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Refinement of anaesthetic protocols for research animals
Key Words	Anaesthesia, Refinement
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Anaesthesia of laboratory animals should represent a refinement of research methods, as it should prevent pain and distress caused by research procedures. However it is important that the best anaesthetic methods are used. This project aims to develop improved methods of anaesthesia for a range of different species, and suitable for a variety of different research projects.

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)?

Improved methods of anaesthesia would benefit animals used in research, as it would reduce the incidence of side-effects such as prolonged recovery periods, reduced food and water consumption after recovery. Improved methods of anaesthesia would also reduce complications such as slow recovery from the anaesthetic, which can increase the risk of death during or after an anaesthetic. These complications can also affect the quality of scientific data obtained from the animals, so the potential benefits are better science, as well as better welfare for the animals used.

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

The numbers of animals used, as well as the species, will be determined by the need for development of particular anaesthetic methods, but will require no more than 300 rats, 300 mice, 44 rabbits and 44 guinea pigs over the 5 years of the project.

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 animals will undergo an anaesthetic, and some will be allowed to recover. The anaesthesia may be repeated. Some anaesthetics can cause slight pain on injection, or can be unpleasant to inhale, but these effects should only cause mild distress and the animals would rapidly become anaesthetized. These procedures would be classified as mild. A very few animals may need surgery to implant monitoring devices to measure the effects of anaesthesia, or to allow infusion of materials or withdrawal of blood. This could cause pain or infection, but we expect to be able to prevent these adverse effects by administering pain relief, and antibiotics, and by carefully monitoring that these are being effective. These procedures would be classified as moderate. Some animals would receive anaesthetics and would not be allowed to recover. These procedures would be classified as non-recovery. Other animals would recover, so that the longer term effects of anaesthesia could be assessed, and these animals would usually be humanely killed once the study was completed. These procedures would be classified as either mild, or moderate. However, when possible, animals that have not undergone any surgery may be rehomed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Since the project aims to develop improved methods of anaesthesia, this needs to be undertaken in living animals, although some aspects of the work (eg developing new apparatus) can be done without the use of animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

At each stage, the number of animals used would be minimised by:

a) Conducting pilot studies using very few animals (typically 1 or 2). This is often sufficient to determine whether a new anaesthetic regimen represents an improvement over existing techniques.

b) Using statistical calculations to determine the minimum numbers of animals needed to show whether a new method is an improvement on older methods of anaesthesia.

Refinement

Explain the choice of animals 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.

Refinement

The age and species of animals selected will be those that match the animals in which the new information obtained on the project will be applied.

When possible, animals will not be permitted to recover from anaesthesia. When recovery is needed, animals would not normally have undergone any surgical procedure. Surgery (to implant telemetry devices) would only be carried out when no alternative approach could be used, and these animals would receive post-operative analgesia to alleviate pain. All of the animals would receive high standards of care during anaesthesia, for example provision of warmth, and monitoring of body temperature to ensure these measures are effective. Animals would also be carefully monitored to ensure no unintended complications occurred during anaesthesia, for example respiratory distress because of inadvertent obstruction of the airway.

PROJECT 65

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Physiology of protein S-acylation
Key Words	S-acylation, endocrine, pituitary, physiology
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The ability of our bodies to respond appropriately to stress is essential for lifelong health and wellbeing and is dependent upon the communication between the brain, endocrine glands and other organs. The aim of this project is to understand how this communication is controlled and in particular how a signalling pathway that modifies cellular machinery by adding fat to proteins controls our physiology in health & 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 short term these studies will provide important new insight into how the stress response is controlled, how it may go wrong and the role of this fatty modification in health and disease. In the longer term this will be of significant benefit to both human and animal welfare as well as providing new therapeutic strategies to tackle stress and related disorders that cost the UK economy £6 billion p.a.

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

A maximum of 6500 animals (rats and mice) will be used during the five years of this project. More than 80% of these animals will be accounted for by the breeding and maintenance of lines of genetically modified mice. We expect that up to 400 rats and 2000 mice will humanely sacrificed to provide tissue for in vitro experiments. The remaining mice will be used for investigations of mild stress responses and physiological responses to changes in diet and environment.

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

The vast majority of animals (>85%) will be accounted for by generating genetically altered animals and subsequent killed for isolation of cells/tissues for experiments in a dish. However, to understand the stress response and stress-related disorders requires the complex interaction between the brain, pituitary and adrenal glands and thus some animals will be exposed to mild stress, changes in diet (e.g having a high fat diet) or sampling blood analytes. The vast majority (>95%) of all such procedures have no adverse effects or have a mild severity. A small number of experiments have a moderate severity for example those requiring surgery, for example to introduce a cannula to sample blood, implant a probe into the brain to stimulate or monitor neural activity, or implant a telemetry device to remotely monitor blood pressure, heart rate and other body functions.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Understanding physiological function relies on complex interactions between different cells, tissues and organs, as well as with effects of diet, stress and ageing. Although the majority of this project will use isolated cells/tissues in a dish along with computer models many of the basic questions we are addressing require us to study the physiology and behaviour of the whole animal. For example, how does the animals response to stress change with different diets?

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We exploit computer models to first make predictions and thus allow us to design the most rigorous experiments for the question to be asked before using animals. This allows us to definitively address a question and make clear conclusions while minimizing the number of animals used. We are also using specific and high resolution approaches that allow us to assay simultaneously multiple body functions in the same animal. For example, by measuring blood pressure, temperature and activity of the animal using devices similar to those used in hospitals for patients.

Refinement

Explain the choice of animals 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.

Refinement

Mice and rats are the species of choice for the experiments in the project as the physiology we are addressing is conserved in mammals, including ourselves, but not in simpler model organisms like worms, fish or flies. Mice and rats are genetically very similar to humans, display physiological responses that correspond in many ways to those found in humans, and have anatomical organization of the underlying physiological pathways (neuronal and endocrine systems) that are also very similar to humans. Several approaches are used to minimise welfare costs to the animals including: i) use of analgesia after surgery and warming and drying animals after a swim in cold water, ii) use of modern techniques to carry out genetic manipulations of restricted groups of nerve and endocrine cells minimizes potentially harmful or confounding effects that may be introduced through global genetic or pharmacological manipulations, iii) many aspects of animal physiology and behaviour will be studied without causing animal suffering, for example by watching animals walk and run, iv) several aspects of the project will include exposing animals to normal physiological challenges such as changes in diet and mild stress – something we all experience for example by eating fatty foods or sitting an exam.

PROJECT 66

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Advanced development of a safe and effective Rift Valley Fever vaccine for livestock
Key Words	Rift Valley Fever, Vaccine, Livestock
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

There is an unmet need for safe, effective Rift Valley Fever vaccines that can be used in pregnant animals. For instance, despite being in use for several decades, the most widely available licensed livestock vaccine (termed ‘Smithburn vaccine’) is contraindicated in pregnancy due to its residual virulence. In this project we will evaluate the safety and efficacy of of a novel promising vaccine called ChAdOx1 RVF in pregnant sheep, goats and cattle, and identify an optimal vaccine dose for its further evaluation in large field trials in East Africa. ChAdOx1 RVF has already been shown to confer 100% protection in non-pregnant livestock in Kenya. Toegther with the safety and efficacy evaluation in pregnancy, and the field trials, the data generated during this project will allow application for registration of ChAdOx1 RVF for use in disease endemic settings in Africa and other regions vulnerable to RVF outbreaks.

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)?

RVF causes high rates (>90%) of mortality in young sheep, goats and cattle. Although older animals are more resistant to disease, high rates of abortion (so-called ‘abortion storms’) are observed following RVF viral infection in pregnant animals and this is often used as a warning sign of imminent human disease epidemics. Humans acquire RVF from infected animals, resulting in an acute self-limiting febrile illness, with occasional severe and fatal manifestations. No vaccines are available for human use and those used in livestock are unsafe for use in pregnant animals. This project aims to address these unmet needs by testing the safety and efficacy of a novel vaccine termed ChAdOx1 RVF in pregnant livestock to underpin its future registration for veterinary use. This will be the first time that the

vaccine platform has been used in pregnancy. The data generated by the study will be useful for the ongoing parallel development of the vaccine for human use

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

Our study will be conducted in pregnant sheep, goats and cattle. We estimate that we will use 30 animals per species, with the entire study lasting 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?

Following inoculation of animals with RVF virus we expect that animals without protective immunity (conferred by vaccination) will develop clinical signs of RVF. These include fever, poor appetite and abortion in those that are pregnant. The main aim of the study is to demonstrate that ChAdOx1 RVF vaccine can provide protection against these adverse effects. All animals will be euthanized at the end of the study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

There is currently no other way of testing vaccine efficacy against RVF-associated abortion and disease, other than live RVF viral challenge of susceptible pregnant animals. However, during data analysis we will seek to identify features of the immune response generated by the vaccine that can be used to predict vaccine performance without the need for viral challenge. Such information may inform the design of future livestock vaccine efficacy studies

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use statistical tools to determine the minimum sample size that allows determination of our study endpoints with appropriate power. The NC3Rs Experimental Design Assistant (EDA) will be used where appropriate for each study

Refinement

Explain the choice of animals 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.

Refinement

There is currently no in vivo or in vitro model that can predict vaccine efficacy against RVF in pregnant sheep, goats and cattle. We will therefore use these target species in our study, and monitor occurrence of pre-defined humane endpoints based on the expected adverse effects associated with RVF. Animals reaching these endpoints will be euthanized to minimise suffering

PROJECT 67

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Axon glia interactions and the assembly of axonal domains
Key Words	Multiple Sclerosis, CMT disease, myelination, demyelination.
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Most of the nerves in the brain and spinal cord of humans and other vertebrates are surrounded by an insulating material called myelin. The cells that make the myelin sheath are called oligodendrocytes in the brain and spinal cord (the central nervous system) while Schwann cells do the same job in the rest of the nervous system (peripheral nervous system). Myelination is crucial for rapid electrical communication because specialized gaps between the myelinated nerve segments called nodes of Ranvier have high concentrations of channels that are essential for transmitting nerve impulses.

When myelin is destroyed, as in multiple sclerosis (MS) in the central nervous system, or Charcot-Marie-Tooth (CMT) disease in the peripheral nervous system the speed of nerve conduction slows because sodium channels diffuse away from the node. Furthermore, without a myelin sheath the nerves start to degenerate for reasons that are still very poorly understood. It is believed that nerve degeneration is linked to the disruption of the nodal sodium channels, and their dispersion is also believed to be at the root of other distressing aspects of these diseases such as pain. There are about 80,000 people in the UK with MS and about 30,000 with CMT. Thus far there are no cures.

REDACT

We are planning to answer these questions by studying myelination in living tissues and cells taken from animals killed humanely using advanced microscopy techniques. It is now possible to observe the key nodal proteins once they have been labelled with a fluorescent tag and identify their location and movement over periods

from hours to days in living tissue in which myelination and demyelination can be observed.

We believe that this will allow us to determine how these vital proteins reach the nodes of Ranvier and what mechanisms promote their stability.

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

We expect that we will gain important new insights into the behavior of these key proteins of the node of Ranvier which will allow us to determine what happens to sodium channels in demyelinating disease and why. Since there is continuing interest in sodium channels as targets for drugs in demyelinating disease to either limit the degeneration of nerve fibres and/or mitigate distressing symptoms such as pain, we believe the outcomes of this work will be of great interest to those who are attempting to develop such drugs; indeed it may be that such drugs might be more usefully targeted towards the proteins that interact with sodium channels.

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

We expect the approximate numbers to be used over the next 5 years under the following headings: 1. Wild-type and transgenic mice expressing fluorescent fusion nodal and paranodal proteins used to study protein trafficking in explant or tissue culture. Approximately 12,000 2. Rats from which neurons, Schwann cells and oligodendrocyte precursors will be prepared. Approximately 600

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?

Expressing fluorescent fusion proteins in our transgenic mice will have no adverse affects. Mice in which we have either performed peripheral nerve crush experiments or inactivated specific genes involved in myelination or axonal function will be monitored carefully and from previous experience we do not expect the level of severity to exceed moderate. Animals exhibiting any unexpected harmful abnormal phenotypes will be killed, or in the case of individual animals of particular scientific interest, advice will be promptly sought from the local Home Office. At the end of each experiment or at the end of their breeding life animals will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The mouse is currently the only mammal in which it is feasible and practical to perform transgenesis on the scale necessary for this work, at reasonable cost. Only mammals have a sufficiently developed Central and Peripheral Nervous system and immune-system to permit ready comparison with humans.

Extensive use of in vitro myelinating co-cultures will significantly reduce the need for more extensive animal use.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The proposed experimental designs and methods of analysis have been discussed with statisticians (and fellow collaborators). Otherwise, we will use the least number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from the literature).

Rat oligodendrocytes and Schwann cells are obtained in much greater numbers per animal, and they myelinate in culture more efficiently than their murine counterparts so the use of rats reduces the number of animals we use.

Rat oligodendrocytes and Schwann cells respectively will be grown in tissue culture immediately after isolation to generate large numbers which, in the case of Schwann cells, may then be frozen down for future multiple use. Although this has never been reported before, in preliminary experiments, we believe that this may also be possible for oligodendrocyte precursors which would reduce the number of rats required.

Refinement

Explain the choice of animals 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.

Refinement

We are using genetically modified mice in order to discover the functions of the genes that regulate the development of the fast nerve communication typical of vertebrates, including humans.

In general, the severity level is Mild but there are some occasions when it is necessary to allow GA animals to develop further so that the phenotype (generally demyelinating, causing some shaking but not preventing access to food and drink thus modeling Charcot-Marie-Tooth disease in humans reaches a moderate severity level where we can assess the full extent of the degeneration,

A critical issue in peripheral nerve diseases such as Charcot-Marie-Tooth disease is how well damaged peripheral nerves can repair-which is normally a fairly efficient process. In order to determine the ability of mice (and humans) carrying specific mutations that model Charcot-Marie-Tooth disease in humans to repair their peripheral nerves the best mouse model is that of nerve crush. Mice with such lesions generally recover quickly and can eat and drink freely 24 hours after crush andanimals will not normally go beyond a slight tremor. They normally recover within 2-3 days and fully recover by 8 weeks after peripheral nerve crush. Analgesia will be used to minimize post-operative pain.

PROJECT 68

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Flow, pulse waves and transport in blood vessels
Key Words	artery, atherosclerosis, heart disease, stroke, biomechanics
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The chief scientific unknown are the reasons why atherosclerosis, the disease underlying most heart attacks and strokes, affects some locations in our arteries but not others; this will be investigated with special focus on the roles of blood flow and the transfer of cholesterol from the blood into the artery wall. A subsidiary aim is to develop new non-invasive methods for the stratification of cardiovascular risk and diagnosis of cardiovascular disease in people.

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

The identification of global risk factors for atherosclerosis, such as hyperlipidaemia and hypertension, has led to the development and widespread use of statins and antihypertensive drugs, and has thereby reduced heart attacks and strokes. In the same way, therapies and benefit could result from identifying local risk factors. Non-invasive methods for stratifying and diagnosing people would also reduce the economic and human costs of cardiovascular disease

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

Rabbits – 750 Mice – 250 Rats – 200 Most experiments will be completed within one a few hours. Some experiments (e.g. involving modification of arterial properties followed by assessment of those properties) will last a few weeks. Occasional experiments involving induction of arterial disease require the animals to be on e.g. fat rich diets for up to 6 months with up to a further 2 months to modify and determine arterial properties.

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 procedures will involve harvesting of tissue or administration of harmless tracers and are classified as mild. A few involve modification of vascular properties e.g. by nephrectomy and are classified as moderate. The potential adverse effects are loss of appetite or thirst, or poor recovery from and sequelae of surgery (infection, failure of wound closure, etc). Signs that intervention is required vary with species but include: inadequate food intake, weight loss, reduced mobility, hunched posture, ruffled fur and unresponsiveness. Animals will be humanely killed

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

An intact cardiovascular system is required in order to study the development of arterial disease and the role of factors such as the flow profiles produced by the heart. We do model flow mathematically, using numerical methods, but the boundary conditions have to be obtained and the solutions validated by using in vivo measurements. We also examine permeability in cultured endothelial monolayers and in perfused vessels, but permeabilities are artefactually raised in vitro so again, any mechanisms that are elucidated in this way have to be checked against in vivo measurements.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Minimum numbers for experiments will be ensured by the choice of appropriate experimental design and the use of power calculations to determine sample sizes. The work will be carried out in accordance with the NC3Rs ARRIVE guidelines. For example, animals will be allocated randomly to experimental or control groups, and non-automated measurements will be conducted using blind protocols. We have developed novel statistical techniques to reduce false positives when comparing maps of, say, disease and flow within arteries, increasing the rigour and reproducibility of out conclusions.

Refinement

Explain the choice of animals 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.

Refinement

Rabbits will be used for most experiments because they have patterns of disease that resemble those occurring in people. Rats and mice will be used for experiments to look at fundamental arterial properties that are unlikely to vary between mammalian species. Our methods generally involve very little harm – they chiefly involve non-invasive measurements, administration of substances in non-distressing doses and humane killing. Some experiments do involve recovery surgery and for these harm is minimised by careful monitoring of animals and treatment with pain killers during recovery.

PROJECT 69

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The testing of bone scaffolds in sheep
Key Words	Bone repair, Scaffold, Stem cells
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

In this study we will test the ability of a biodegradable polymer based scaffold to encourage bone growth. This will be tested in a bony defect in the knee of sheep. The material to be tested is a 3-dimensional (3D) scaffold with a high number of linked pores, which should allow cells to migrate through the defect whilst providing support for the bone to grow. In addition the scaffold will be coated in substances which encourage bone to grow and have stem cells added which should increase the rate of bone growth.

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)?

Worldwide there are a large number of surgeries carried out that require bone grafting, which is classed as the gold standard and therefore serves as a point of reference against which other bone graft materials may be compared. This can be due to fractures, osteoarthritis and osteoporosis, which are more prevalent with an aging population. Bone grafting has significant risks associated with it, for example rejection and infection, multiple surgeries, increased hospital stays and greater demand and costs put on the NHS. There is also usually a lack of sufficient bone graft, therefore donor bone may be needed. The material we are working with will hopefully be able to support bone growth, cell migration and the formation of blood vessels within a defect site, and could benefit humans. This in turn should reduce the number of surgeries and infections associated with bone grafting and provide a material which encourages better bone fill to a defect site.

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

76 sheep over 5 years

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

We expect that the animals will be slightly lame for a couple of days and then will have no adverse effects of the surgery. In addition the animals are generally standing and eating in about 1 hour post-op. Animals will be humanely killed to allow us to harvest the knees and investigate the amount of new bone that may have grown into the defect site. We will use micro-computed tomography and histology to look at the quality and the amounts of new bone within the defect.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Cell based studies are useful to look at how cells will behave in contact with the scaffold, however cell assays alone cannot adequately model the complete array of effects important in bone modelling or repair. Using stem cells we have shown that cells were viable, proliferated, and were evenly distributed throughout the scaffold, suggesting that the material would not be toxic and encourage bone growth. Predictions of degradation rates and pore numbers have been obtained from these studies for varying formulations; however none of these assays can adequately model the *in vivo* environment of a whole animal. Therefore we need to test the material in an animal model before it can be translated into use in a human.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use both hind legs in an animal to reduce the overall number of animals used. Statistical analysis has been employed to calculate the minimum number of animals we could use and still have a scientifically relevant study. This value is a minimum of 6 defects per experimental group. Data will be analysed using a suitable statistical package and statistical tests, for example one way analysis of variance and post-hoc testing. All experiments will be conducted in a manner that will allow high quality publication.

Refinement

Explain the choice of animals 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.

Refinement

The use of REDACT in orthopaedic research is increasing. REDACT are useful models as they have a similar bone and joint structure, body weight and have a comparable rate of bone remodelling as humans. The model involves initially aspirating cells from the sternum of a skeletally mature female REDACT and culturing them, before creating a bony defect to return them within the scaffold material. All surgery is carried out aseptically in dedicated facilities with experienced staff. Control defects to compare the scaffold's success to will be created using either bone graft from the sheep or leaving the defect empty. Control sheep will only undergo a single anaesthetic event.

All animals will receive pain relief during and after surgery. Antibiotics will also be given to prevent infection. After complete recovery from anaesthesia the animals will be returned to group housing. From previous studies we do not expect the animals to be lame for a significant period of time, but if they are unable to stand or not showing signs of improvement they will be killed to prevent any suffering. A scoring system will be used to monitor the animal's well-being after surgery.

PROJECT 70

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Ligament repair
Key Words	ligament
Expected duration of the project	1 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The work to be carried out under this project licence will be done to improve the experience of patients undergoing ligament surgery. It also aims to reduce costs for the Health Service due to shorter stays in hospital. Currently there is a large unmet need for developing technologies and products to enhance ligament surgeries that reduces pain and suffering to patients and decreasing the financial burden on the healthcare system. These technologies and products will help patients worldwide to regain normal lives in terms of their ability to carry out everyday functions which had been prevented either by degenerative joint disease or by trauma. The economic benefits would include fewer days lost at work, fewer hospital days and reduced care costs.

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 benefit is the evaluation of safety of new types of materials to be used in orthopaedic surgery.

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

This licence propose to use sheep, rabbit and goat models and the number of animals will not exceed 500 per protocol.

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

The surgical procedures detailed in this licence will cause mild or moderate post-operative discomfort which will be controlled by analgesics and refinements made

from our previous experience. The maximum level of severity will be moderate. The animals will be killed at the end of the protocols.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In vitro testing cannot fully replicate the *in vivo* loading, physiological and anatomical conditions required to demonstrate safety and efficacy of novel fixation devices, therefore animal studies are necessary in the development of new fixation devices.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Consultation with a biostatistician at the planning stage will be actively used to optimise study design, minimise the number of animals required, and meet the study objectives.

Refinement

Explain the choice of animals 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.

Refinement

A thorough investigation into the most relevant species to use for these proposed animal models has been conducted in the previous project licence for ligament repair.

The majority of protocols utilise REDACT? as this species has bones of the size that will allow implants suitable for humans to be used. We have a great deal of experience with many of the protocols described in this licence. This experience has led to refinements in surgical technique, analgesic regimes and post-surgical care.

PROJECT 71

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Myocardial function, metabolism and protection
Key Words	Stress-induced cardiomyopathy, Imaging
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aims are to establish the mechanism in which the energy production in the hearts in stress induced cardiomyopathy (Tako Tsubo) is decreased. To begin with we would like to use a treatment that already exists for angina, to try to improve the metabolism and the function of the heart. If successful this would be trialled in a control clinical trial and would be the first treatment available for Tako and would potentially be a lifesaving therapy. We intend to use imaging techniques such as PET scanning to achieve the aims of the study.

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 above aims and objectives are immediately translatable into the clinical projects, indeed the purpose is upon completion of the experimental data to advance this directly into clinical trials. This condition affects 7% of people who are admitted with a “presumed” myocardial infarction, and affects more women than men. Little is known about this condition.

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

Mice, rats. 490 total. 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?

Most of the protocols are mild and one is severe. Some asymptomatic GA rodents will be used. Substances will be administered to rodents and the heart will be imaged. Animals will be humanely killed at the end of the procedure and the tissues analysed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Heart muscle diseases (cardiomyopathy) are whole organ conditions, integral of blood supply, heart scaffold (matrix) and heart cells, as well the innervation of the heart and these cannot be replicated in animal alternatives.

We are already examining the mitochondria from isolated cells in this experimental set up. However, it would be inconceivable to recommend a treatment in clinical practice on the basis of having explored a therapy in cells alone. The experimental l set up described is a compulsory step into advancing this therapy to a clinical study.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Through high level expertise in all procedures performed precise measurements of end points to increase sensitivity of measurements (this is where high-end cardiac imaging plays a significant role) and robust power calculations done in agreement with a qualified statistician.

Refinement

Explain the choice of animals 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.

Refinement

Mice are a well described model of heart disease and have known phenotypes. For the stress induced cardiomyopathy we use primarily female rats because: the condition is seen in females in clinic (proportion of 9:1 to males) and the rat model replicates very well the human condition with minimal mortality of the model. Staff are experienced in the model and all rodents are closely monitored. A cooling system was developed under the current licence for alleviating the hyperthermia in those animals who develop it in the first few hours after the model is induced.

PROJECT 72

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Understanding proliferation versus differentiation in cells and tissues.
Key Words	Division, maturation, cell fate, cancer
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We need to understand why cells divide and how they chose to stop dividing before maturing into a functional state, so we can manipulate these processes for making new cells for therapy, as well as stopping disruption of these processes in 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)?

We will understand better how the normal nervous system, pancreas and gut form in development and how they maintain themselves in adulthood both at the level of tissue behaviour and by looking at the molecular mechanisms of control of these processes. We aim to enhance the ability of cells in the pancreas, gut and nervous system to adopt and maintain a mature state, which will aid in the treatment of diseases of these tissues where functionality is lost such as diabetes and cancer. Data generated will also benefit other researchers who work on cancer and development. They will be applicable to humans and to animals with these conditions.

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

We will use mice. We expect to use up to 20,000 mice over the 5 years of this licence to achieve our scientific aims.

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

The vast majority of procedures are expected to have no effects on the animals other than giving injections and taking blood samples. In a small number of cases we will

be using mice that are genetically engineered to develop intestinal or pancreatic cancer and these mice may show adverse effects such as weight loss, and diarrhoea. These mice will be closely monitored to ensure that their welfare is not unduly compromised and any suffering is minimised as far as possible e.g. by enhanced monitoring and supportive intervention such as wet food. At the end of the experiments, animals will be humanely killed and tissues will be taken for further analysis.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We are studying how embryos develop and how the tissues that form are maintained in adulthood. The complex network of genetic and cellular interactions that control these processes are, as yet, largely unknown and we cannot use any system other than a living animal to study them. Indeed, animal studies are unavoidable if we seek comprehensive knowledge and understanding of gene function, physiology and pathology. However, where possible we will first undertake similar experiments using tadpoles under a separate licenced programme of work, which will allow us to establish biological principles in living embryos that are not mammals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Prior to embarking on animal experiments we will collect as much evidence as possible to determine whether our experimental hypothesis is likely to be correct. Indeed, much of the programme of work described here is based on [REDACTED] so we already have evidence in frogs that the mechanisms that control cell division and maturation that we aim to study in mice are active in this “lower” species. Some of these experiments will lead on from experiments where we change the genes in cells that we can grow in the lab and see whether this changes their behaviour. We will then need to see whether the same thing happens in mice. We are also using mini-organs which grow independently of the body in culture dishes (organoids) to study as many aspects of cell division and maturation as possible, which reduces and replaces some mouse experiments, although tissue for these mini-organ cultures are originally derived from mice, and each mouse only produces tissue for a small number of organoids. When we have types of genetically modified mice of scientific interest but for which we have no immediate use, we will freeze embryos that can be thawed out at a later date.

When designing the experiments, we perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative. We have many years of experience in determining the correct sample sizes for the sorts of experiments we undertake. To get enough statistical power, we generally need n=3-5 per group for our analysis, and we anticipate looking at, at least, 3 time-points per type of mouse. Advice will be sought from a collaborator who is conversant with the sophisticated mathematical and statistical analysis that will inform our experimental design. Indeed, our experience of analysis of a genetically modified mouse previously studied with another of our collaborators has told us that, in general, 3-5 of each type of mouse per time-point are the minimum that will be required for statistical significance for analysis when we are looking at changes in cell numbers and tissue organisation. We would aim to look at at least 2-3 developmental stages as well as adults, depending on the effects that we see, as this allows us to gain the maximum information about how tissues form during development and adulthood.

To maximise the information from a single animal, we will collect samples from multiple organs and tissues and share these with other scientists who are asking similar questions, so they do not have to breed their own mice for experiments.

We will remain alert to any advances that will enable the replacement of animals.

Refinement

Explain the choice of animals 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.

Refinement

REDACT? which has generated data underpinning much of this programme of work. However, we must now determine whether regulatory mechanisms found in the frog are the same in mammals.

The mouse is the most appropriate animal model for this study because: (i) it is a mammal; (ii) physiology is more extensively characterised in mice than in other mammalian species; (iii) mice are amenable to changing their genes; (iv) a large number of relevant genetically modified mice are already available. The experiments in this proposal will involve creating and analysing genetically modified mice and are classified as mild to moderate with respect to potential discomfort, stress or suffering.

Specifically bred mice will be maintained in a clean environment to decrease the risk of infection. During this project, some mice will receive substances including chemicals that can switch on genes, which normally do not harm mice. Those will be administered mostly by injection, the pain of which is considered to be mild. Alternatively, where possible, these substances will be administered in food and

water, resulting in minimal, if any, discomfort. We are using well-established chemical substances to alter the genes of the mice, so potential bad reactions to the substances used are minimised as far as possible. Taken together the overall harm to mice that can be caused by performing our experimental plan is minimal and the obtainable knowledge and benefit for our society is significant.

We have chosen mice that are designed to develop cancer where the development of tumours is well established. We have safeguards in the form of limits to weight loss and the detection of signs of compromised health such as inactivity, hunched posture and diarrhoea that allow us to act at an early age to humanely kill mice to avoid unnecessary suffering. Moreover, when using new sorts of mice genetically engineered to develop tumours , we will seek training from other scientists who are familiar with how these cancers develop to make sure that we know what to expect and are alert to early signs of suffering.

Where possible, we will use “mini-organ” cultures from mice that can be grown outside the body to investigate molecular mechanisms of control of cell division and tissue formation. These often divide limitlessly in plastic dishes as well as mature into functional tissues when we change the chemicals they are grown in.

PROJECT 73

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Breeding, maintenance and supply of Genetically altered and harmful mutant rodents
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Genetically altered animals (GAA) particularly rodents are going to be bred under this project license to supply to establishment in the UK EU and outside the EU for research into the control of disease, ill health or abnormality and/or the study of normal and abnormal physiology, biology or behaviour. They will be used for the discovery and development of new medicines for the treatment and prevention of human disease. Rederivation or cryopreservation techniques will be used to improve health status of animals or preserve lines if live colonies are not required. A range of transgenic technology services and colony management/breeding services will be provided as requested to other research or scientific establishments.

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)?

Genetically altered animals (GAA) particularly rodents are currently in widespread use in biological, medical and veterinary science and have shown to be of great value in elucidating the function of genes and pathways in a wide variety of biological, physiological and pathological processes.

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

All protocols will be based over 5 years

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

Animals produced under protocol 5 of this project license are not expected to exhibit any harmful phenotype. Records will be maintained with regards to the nature of the

transgene/mutation, genotype, phenotype and any possible effects and specific husbandry requirements. Clinical signs prevalence of incidence, age of onset, if necessary photos will be used in a passport style document to help staff recognize such effects and to plan strategies to alleviate or avoid such effects. Animals with an altered immune status will be maintained in a barrier environment thereby minimising the likelihood of compromising health. Animals exhibiting any unexpected harmful phenotypes will be killed (Schedule 1), or in the case of individual animals of particular scientific interest, advice will be sought promptly from the local Home Office Inspector.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Use of live animals is required for breeding purposes.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Breeding is closely monitored ensuring peak breeding performance is maintained on a month by month basis, breeding will be adjusted according to customer demand, this will ensure wasteage is kept to a minimum. e.g. removing males from pairs to reduce number of litters.

Refinement

Explain the choice of animals 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.

Refinement

Breeding is closely monitored ensuring peak breeding performance is maintained on a month by month basis, breeding will be adjusted according to customer demand,

I will ensure continual training occurs at our facility via the continuous professional development (CPD) program for all staff. This will make sure that we are fully aware of any changes to methodologies in this industry than can minimise animals used or reduce severity of animals bred or on protocols.

PROJECT 74

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Extracellular matrix in development and disease
Key Words	Extracellular matrix, Circadian rhythm, Osteoarthritis
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

1. To understand the function of various components of the extracellular matrix (ECM): the supporting scaffold material for cells, and their daily rhythmic regulation, in its early development;
2. To determine the mechanisms by which mutations in these matrix and body clock (circadian rhythm) genes result in specific diseases, including osteoarthritis;
3. To test potential novel therapies using the mouse models generated in our programme.

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

Advancement of biological knowledge This work will help us to understand how this molecular supporting scaffold is formed and maintained on a daily basis. This information is of relevance and interest to the whole field of ECM. In addition, the knowledge gained underpins our understanding of changes in the connective tissue in disease. Defining disease mechanism and identifying novel treatment opportunities Osteoarthritis is common, affecting 60% of people over the age of 65. Chondrodysplasias (malformation of the cartilage) caused by mutations in ECM genes are relatively rare. There are currently no effective treatments for chondrodysplasias. The only options for osteoarthritis are joint replacement and analgesia. There is an association between these two conditions which affect cartilage cells (chondrocytes) and various forms of abnormalities, such as the endoplasmic reticulum stress (ER stress) and the body clock system. The importance of these findings is that they highlight new potential avenues for the treatment of these conditions.

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

Mice. In total, 10,000 mice are expected to be generated and used over 5 years.

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

Protocol 1: Breeding and maintenance of genetically altered mice. Severity level: Mild Chondrodysplastic strains have a mild dwarfism and do not cause obvious pain or discomfort. We do not anticipate any adverse effects as a consequence of removing relevant genes. A small number of animals will be observed up to 18 months. We do not anticipate any significant age-related side effects in mice up to that age. Protocol 2: Treatment of chondrodysplasia or osteoarthritis. Severity level: Moderate Those animals that undergo anaesthesia on several occasions will only be re-anaesthetised when they have fully recovered from the previous anaesthetic. Protocol 3: Induction and treatment of surgically induced osteoarthritis. Severity level: Moderate Mice will be humanly killed for detailed studies.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We are studying the complex biological process by which long bones grow and by which the articular cartilage subsequently can become degraded in osteoarthritis. We make extensive use of cell culture models. However, the holistic process of bone growth and osteoarthritis cannot be modelled using in vitro techniques. We are now at the stage of directly determining whether endoplasmic reticulum (ER) stress and body clock disruption are pathogenic factors in the causation of osteoarthritis and for these studies, there is no alternative but the in vivo model.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Some of the measures are essentially qualitative. Others are quantitative, requiring statistical analysis.

For qualitative experiments, defined breedings (where all the progeny have the desired genotype) are employed to reduce numbers of animals produced. The

number of observations will be the minimum necessary to provide an adequate description.

For quantitative aspects of the programme, age and sex-matched animals are used. For quantitative assessments of disease severity, a blinded assessment of outcome will be performed. Where feasible, the animals are littermates to reduce variation. Clearly, the exact numbers of animals required will vary with the particular experimental design, and so on.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the ideal model for these studies. Mice have a skeletal system that develops in a similar fashion to humans; they develop osteoarthritis similar to that seen in humans in response to joint destabilisation in a relatively short (8 week) time frame. Thus we have been able to produce genetic mouse models of specific human chondrodysplasias and osteoarthritis.

PROJECT 75

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Maternal diet and cognitive development of the offspring
Key Words	Maternal diet, behaviour,metabolic disease, fetal programming
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Surveys show that Scots from poor socio-economic backgrounds are less healthy. It is our proposition that individually mild nutritional imbalances in the diets of deprived communities combine to have a substantial effect on the development of the unborn babies and infants, adversely affecting the way they learn and behave in later life. We also think that the mother's diet influences the risk of diabetes later in life. This hypothesis will be tested by feeding rats diets which have been formulated to resemble those consumed by pregnant women in Scotland. Some women eat diets which have more fat and sugar and fewer vitamins than are recommended. These diets may affect the learning and health of their babies. We will test this hypothesis in rats by feeding them a diet which accurately resembles the diet eaten by pregnant women. We will allow the animals to breed and then study the behaviour and health of their offspring. Behavioural tests will measure the ability of the animals to learn and test their response to stress. We will also see if there is an increased risk of metabolic diseases such as type 2 diabetes.

Some of the effects of the imbalanced human diets may be a consequence of changes in the DNA of the cell, a process known as epigenetic programming. This permanently changes the DNA, affecting gene expression. We will be looking for these changes in tissues from the animals and seeing if the changes are associated with the diet. Because the changes in DNA are permanent, we believe that there may be effects on subsequent generations. We will breed a second generation from the offspring to investigate the potential for transfer to subsequent generations.

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)?

These studies will provide basic information on how major nutrients (protein, carbohydrate and fat) interact with micronutrients (vitamins and minerals). We will see if individually minor deficiencies in the mother's diet add up to make a much larger cumulative effect. The loss of human potential and the economic consequences of poor adult health are substantial. Policy makers need information on the effects of nutrient imbalances in order to make evidence based recommendations. An understanding of the interactions is important in knowing which nutrients to focus in in health policy.

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

This study will use rats. We will use about 100 animals for the initial studies of maternal and fetal metabolism. A further 500 offspring (assuming 10 per litter) will be used for the postnatal studies. Some of these animals may be bred to produce another 500 offspring in the second generation

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

We will be feeding animals a nonstandard diet and measuring the effects via blood samples, scanning, and measurements in the tissues after death. We will study the offspring with simple maze type behavioural tests, scanning, blood samples and blood pressure measurements. The experiments fall into the mild severity range. The adverse effects are limited to minor discomfort. The behavioural studies will not produce adverse effects.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

There are serious ethical and experimental constraints on studies in human populations. There is a complex metabolic relationship between the mother, fetus and infant. However it is very difficult to intervene in human pregnancy. It is also difficult to separate the effects of a poor diet from other effects of deprivation such as poor housing. The animal model eliminates effects which otherwise confound human studies and will provide us with information on the metabolic effects of combined nutrient imbalances. These studies will complement a large study of human volunteers but will give us access to tissues like the brain which are very difficult to obtain from humans. The duration of the human life span places severe restrictions upon the analysis of early-life nutritional interventions in adult humans, frequently effects can only be inferred from changes in putative biomarkers. These studies are

designed to parallel ongoing studies of human cohorts, providing information that cannot otherwise be obtained.

Cell culture models lack the complexity of the animal, making it impossible to study the full range of interactions in vitro, although cell cultures are being used in preliminary experiments to investigate specific molecular mechanisms.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Before starting each experiment the study proposal is scrutinised by a statistician in order to ensure that we are using an appropriate experimental design. Power calculations are carried out as a matter of routine to ensure that we have an appropriate number of animals to achieve our goals. In the postnatal studies we will minimise the use of animals by cross fostering surplus pups to dams with smaller litters.

Refinement

Explain the choice of animals 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.

Refinement

The behaviour of the rat is well known and the behavioural tests are well established and recognised. We have been studying nutrition and reproduction in the [REDACTED] Our experience provides us with extensive data sets of previous data on factors such as animal growth, food consumption etc. We already have a substantial body of information on the metabolism of the pregnant rat and this study will build on this information. All studies will be carried out by highly experienced staff and veterinary advice will be sought whenever necessary. Additional advice will be sought from [REDACTED].

PROJECT 76

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Nutritional programming of metabolic disease
Key Words	Suboptimal nutrition, pregnancy, early life, programming, adult health
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Our overall purpose is to identify the mechanisms leading to adult disease, secondary to suboptimal maternal nutrition during pregnancy and/or lactation, and to investigate the efficacy of interventions that are potentially translatable to humans.

The key questions are:

1. **How does suboptimal gestational and/or lactational nutrition affect maternal and offspring health and what are the underlying mechanisms?**
    - What are the key maternal factors (e.g. hyperinsulinemia, hypertension) in an obese mother that program metabolic fitness, cardiac dysfunction, energy balance in offspring?
    - As proof of principle, can we mimic these programming effects (e.g. insulin) by introducing these maternal factors directly into the fetuses?
2. **What are the effects of pharmacological and/or exercise intervention to the mother, and the metabolic health of her offspring?**
    - Does perinatal maternal exercise in an obese mother improve her metabolic and cardiovascular fitness?
    - Does perinatal pharmacological treatment improve her metabolic and cardiovascular fitness?
3. **What are the effects of dietary/pharmacological intervention in the offspring on the programmed effects on metabolic health?**
    - Does perinatal maternal exercise in an obese mother improve metabolic and cardiovascular fitness of her offspring?
    - Does perinatal pharmacological treatment in an obese mother improve metabolic and cardiovascular fitness of her offspring?

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)?

Lifestyle (increased physical exercise) intervention to an obese mother around the time of pregnancy is likely to improve her metabolic fitness and the long-term health of her offspring. This is an important translational message.

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

Over the 5-year period of this project, we expect to use no more than 6,900 adult and 7,500 neonate Rats; 11,600 adult and 10,400 neonate Mice.

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

The large proportion of animals to be used in this licence (81.9% of the adult rats and 87.5% of the adult mice) will experience no adverse effects. Female animals fed differing diets, with or without exercise or pharmacological intervention, will then be paired with a male. Both mothers and offspring health will be monitored throughout adult life using longitudinal assessments of a) non-invasive cardiovascular imaging techniques, sometimes with a general anaesthetic to negate stress b) metabolic testing involving All animals will be killed humanely at the end of the experiment. 15.2% of the adult rats and 10.8% of the adult mice will undergo surgery under appropriate general anaesthesia. Following surgery, they will experience minor discomfort with itching around the wound stitches. This will be managed with appropriate analgesia and antibiotics. Animals will be killed humanely at the end of the experiment. 2.9% of the adult rats and 1.7% of the adult mice will undergo surgery under general anaesthetic throughout the experiment and at the end of the experiment, they will be killed by an anaesthetic overdose.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Although experiments in cultured cells can provide a lot of useful information on specific workings at a cellular level, we need to study how different cell populations behave and interact as part of a complex environment in a living animal. Each tissue type e.g. brain, fat tissue, heart, working muscle or liver, are themselves made up of different cell populations (including stem cells which go on to divide and mature into fully functioning adult cells). The different tissue types send out and respond differently to the signals present in the peripheral blood system such as occurs in the whole living animal. This level of complexity cannot be attained in cell culture based experiments.

Our previous work has used the strategy of identifying specific mechanisms due to suboptimal maternal environment in animals which lead to cardio-metabolic disease in the offspring, and then using this information to guide parallel human studies (e.g. in Danish low birth-weight men). This successful strategy clearly underlines the value and justification for our work using animals, which has significant parallels with the human pathophysiology. We will where possible, use a similar approach to translate our observations in animal models into humans by working on human serum and biopsy material and we will continue to use a forward and back translation approach.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

It is important to note that as the environmental stimulus is made to the mother, it is the mother which becomes the statistical unit for all our studies, and this is therefore reflected in our animal numbers as we are constrained to using only 1 offspring in each litter for any given outcome. We will collect all tissues and organs at post mortem-even ones that are not required at the time for any particular study. These include brain, heart, aorta, lungs, liver, pancreas, fore-gut, hind-gut, kidneys, intraperitoneal fat, retroperitoneal fat, gonadal fat, vastus lateralis & biceps femoris muscle, brown fat, bones and testes or ovaries. This extensive bank of tissues allows us to follow up several lines of disease pathology, so that we and other groups through internal and international collaboration, are able to facilitate later studies without the need for additional numbers of animals, thus significantly reducing the need to use more animals.

Where possible we will follow up observational studies in the animal with non-animal cell systems to gain mechanistic insight into specific cell signaling pathways. These studies will help define the specific pathways involved and thus inform specific intervention, resulting in a reduction in the number of animals and a high degree of refinement to the proposed intervention models. For example, we observed that maternal obesity “programmed” a loss of IRS1 (an insulin signalling molecule) in the fat tissue of the offspring of obese mouse dams. In the same tissues, we also observed a gain in the expression of a small RNA molecule (microRNA) known to negatively regulate IRS1 protein levels. In order to investigate if these programmed changes could be replicated in a cell system, we obtained precursor cells from the fat tissue of these mice and grew them into mature adipocytes in vitro. This experiment showed us that despite being grown and outside the animal, these precursor cells carried the information encoding the programmed phenotype observed in the mature fat cells of the animal. This strategy will allow us to **reduce** animal numbers further by adopting a cell system widely used in studies of regulation of fat metabolism for our more complex studies. We will also couple the use of this cell system with a global approach to identify other proteins regulated by the

overexpression of any microRNA in a non-biased manner and without *a priori* bioinformatics prediction.

We will use non-invasive echocardiography with recovery anaesthesia to monitor cardiac function longitudinally, in the same animal that **reduces** the number of animals required. Isoflourane is very well tolerated in every animal as we maintain anaesthesia for no longer than 20 minutes and recovery is quick (under 1 minute). This provides substantial gain in power and data quality and robustness thus reducing animal numbers.

Refinement

Explain the choice of animals 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.

Refinement

Females from our breeding colony will be randomised (by animal technicians blinded to the study) to receive experimental diets and or interventions. At sexual maturity, they will be mated with male studs that are refreshed every 6 months to optimise breeding and minimise ageing paternal effects on the offspring. Offspring are again randomised (by technicians blinded to the study) to be weaned onto control or obesogenic diets with or without pharmacological intervention. Where possible, investigators carrying out cardiometabolic measurements will be blinded to the experimental groups.

In our exercise intervention studies, we apply the knowledge that rodents are nocturnal and therefore they respond better to exercise training at the beginning of their wake cycle (which is after 6pm in the evening when the lights are out). Our researchers therefore go in to train the animals in the dark with a red headlamp to minimise disturbances to their circadian rhythm.

We use non-invasive TDNMR that does not require anaesthesia to measure body composition of mothers and offspring. As this allows longitudinal body composition to be measured in the same animal, it also reduces the number of animals required. We combine this data with other repeated longitudinal measures such as non-invasive tail cuff blood pressure and Echocardiography. Finally, at post-mortem, we take blood to measure metabolites and tissues for molecular studies. All the data can be correlated and then also compared to aged controls to identify if there is an advanced aging phenotype. From this, we can identify markers at the cellular level indicative of ageing, which enables us to assess the effects of early nutrition on life span without the need for maintaining mice and rats for their full lifespan. During the next 5 years, we hope to acquire access to more advanced imaging equipment that would allow in-utero measurements and measurement of ECG in conscious animals.

PROJECT 77

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Development of basal ganglia circuits
Key Words	Development, Brain, Basal Ganglia
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Many aspects of general brain development are unknown. The basal ganglia, a deep brain structure important for motor control, is no exception. For example, it is unknown what type of information is of particular importance during its development. Newly generated neurons contain the genetic instructions from their parent cells but are also exposed to activity patterns from surrounding neighbouring cells during their early life. The main objective of this proposal is to untangle the relative importance of these two sources of information. This will generate a blueprint of neural circuit development with which to compare these processes in neurodevelopmental disorders (e.g. Tourette's syndrome, OCD among others), which form a burden to society.

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 proposed programme of research aims to elucidate a fundamental question in neuroscience, which is how genes and early activity patterns shape the development of neurons and neural circuits. The beneficiaries of this proposed programme of work are several. It will increase our understanding of basal ganglia development, which will benefit other researchers trying to understand basic questions on brain development in health and disease. It will benefit patients in that it aims to provide new targets and rationales for treatment of neurodevelopmental disorders.

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

This proposed programme of research will use both wildtype and transgenic mice and rats. Over the lifetime of this licence all effort will be made to minimize the numbers of animals used. Our licence application estimates that we use no more

than 11,500 mice and 2070 rats over the lifetime of the licence and most likely the number of animals used will be significantly smaller.

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

We will be breeding a substantial number of animals of which the majority are well characterized and will not exhibit any adverse effects and therefore be bred under a mild protocol. Some new transgenic lines will be used and if an animal does exhibit adverse effects a plan is in place to manage and alleviate these and the animal will be moved to a moderate breeding protocol which includes increased monitoring. The mice can undergo three types of procedure in vivo. Firstly, the administration of substances (e.g. to label cells or activate genes) to prenatal and postnatal animals under appropriate anaesthesia, analgesia and aseptic surgical techniques. Appropriate plans of monitoring and analgesia are in place to deal with any potential discomfort or pain and will be expected to be of moderate level. Secondly, the recording of brain activity in prenatal and postnatal animals. This will be done under terminal anaesthesia and is classified as non-recovery. Lastly, the observation of behaviour of animals. This might involve some stress to the animal, but this will be monitored and refined and this is expected to be of moderate level. All animals will finally be killed using Schedule 1 procedures or terminal anaesthesia and used for either electrophysiological (in vitro) or immunocytochemical analysis.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Neurons are not present outside the animal kingdom, so an animal is required. There is no alternative that would entirely replace the use of a living animal that would allow all of the objectives to be met. Although computer models have provided great insights into neuronal network behaviour in recent years, the models currently available in the field are not yet sufficient to address our key objectives here as many of the basic variables are still unknown to us (e.g. connectivity between neurons, activity patterns in certain brain regions). Cell culture systems, which provide large benefits to other areas of science, do not recapitulate the full gamut of neuronal activity and often form aberrant synaptic connections. As such, the proposed project necessarily involves experiments on animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used will be kept to a minimum at every stage of the project and by several means. Firstly, by careful planning of breeding and experiments (e.g. power analysis) in close contact with staff and animal technicians we will minimize animal numbers. Secondly, the designs of our experiments are such that we maximize the usage of tissue and minimize the number of animals. E.g. a single animal can be used to generate 12 brain slices and each brain slice can be used to record 4 neurons allowing the recording of up to 48 neurons from a single animal. Any surplus slices can furthermore be used for immunocytochemical studies. Thirdly, we will use animals from all genders and both homozygotes and heterozygotes wherever possible. Lastly, we will techniques to genetically alter single animals, which will alleviate the need to generate new genetically, modified mouse lines, which typically require the breeding of many generations of mice.

Refinement

Explain the choice of animals 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.

Refinement

Rats and mice have been chosen as details about their central nervous system have been well documented and they are the lowest form of mammal that can provide meaningful data about man. Most studies will employ normal adult healthy animals. Occasionally, genetically altered animals may be used to model specific CNS disorders or provide proof of concept for novel targets for treatment of CNS disorders. Where we are performing recovery surgery this will be performed aseptically and under appropriate anaesthetic and analgesic regimes.

PROJECT 78

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Diet Induced Obesity model in rodents
Key Words	Obesity
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This is a licence in which the programme of work is designed to produce prepared rodents and/or use of rodents to conduct basic early stage research. The rodents form the basis of several different animal models of diseases such as such as type 2 diabetes, high blood pressure, high cholesterol and other diet induced circulatory problems.

Essentially animals are fed a high fat diet or high fat/high sugar diet for a number of weeks. As a result, they become obese, and usually have high blood sugar, and develop impaired sugar tolerance. These animals are then used to study the adverse clinical and genetic effects of obesity and type 2 diabetes and to test drug candidates that are expected to affect these conditions in humans.

This is a demand-led service supported by a scientifically justified need. We will never exceed the moderate severity limit.

The scientific background for each individual use is specific to each researcher but broadly addresses scientific and clinical needs in the following areas of research: obesity and metabolic related disease.

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

The benefits of the service program are therefore to provide facility for the scientific community to complete these programs of work where resources may otherwise have been limited. The availability of appropriate facility and expertise then ensures the procedures will be performed to a consistently high standard allowing greater reproducibility and ensuring the highest standards of rodent care. The benefits of this project are principally (i) the control of the number of animals being bred and developed, due to the ability to plan better due to greater demand (ii) good

understanding of the model by the staff looking after them, potentially resulting in better animal welfare.

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

The numbers of animals used is not expected to exceed 5000 mice and 2000 rats over 5 years.

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

There are several potential outcomes at the end of the protocol: shipped to the client for further study; prepared and kept at our Establishment to continue onto approved early stage experimental studies: or placed directly on early stage experimental studies. Most of the animals are expected to experience no more than mild clinical signs associated with obesity. A small number are expected to develop clinical signs due to the development of diseases such as diabetes or dermatitis and these animals may experience moderately severe adverse effects, which may be controlled by special care, veterinary treatment or by humanely killing the animal if it appears to be developing adverse effects which are worse than predicted. Where live animals are not required by the researchers, collection of bloods and tissues following immediate humane killing or after induction of terminal anaesthesia will be performed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animals are essential for research into human and animal diseases, as behavioural models and full system responses cannot be replicated using non-animal methods.

Our Establishment will make consideration to the use of alternatives for every study and if unable to replace the use of live animals, will identify the most appropriate reduction strategies for the research work.

Our AWERB (Animal Welfare and Ethical Review Body) will assess the project proposal internally for all new diet induced obesity (DIO) clients/protocols to ensure all projects have good scientific and ethical justification and the study design is sound and meets the 3R's and scientific outcome required by the customer. No new projects will begin without the full approval of the AWERB in line with the Home Office licence.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Consideration to identifying the most appropriate ways to reduce the numbers of animals used for their research work will be discussed with every new client project enquiry.

Our AWERB will also assess the project proposal internally for all new DIO clients/protocols.

Recommendation will be made to reduce the number of animals used to the minimum required to safely deliver a valid result, use of pilot studies to define the optimum schedules for induction/treatment will be considered.

Refinement

Explain the choice of animals 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.

Refinement

has taken consideration into identifying the most appropriate refinement strategies for their research work. [REDACTED]

Anaesthetics and analgesics will be implemented on advice of the vet to minimise cost to the animals.

Our AWERB will also assess the project proposal internally for all new DIO clients/protocols.

Animals will be housed in bio-secure enclosures/rooms to ensure a high health status and routine health testing will be in place; both to ensure their wellbeing and to ensure that experimental outcomes are consistent and therefore use a minimal number of animals.

PROJECT 79

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Cancer progression and Metastasis
Key Words	Cancer, Metastasis, Microenvironment, Therapeutic response, Cell biology
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

In the UK, >11,000 women and men die of breast cancer each year. The majority of these deaths result from the spread of the cancer to secondary sites in the body, the process known as metastasis. The spread of cancers involves a close interaction between the cancer cells and their microenvironment - the normal cells and matrix components of the body. The aim of this project is to identify novel strategies for preventing or limiting the development of metastatic disease. To achieve this we have two objectives (1) to identify how the cancers cells interact their microenvironment as they leave the primary tumour and spread to other sites in the body, and (2) to understand how these interactions can impair the effectiveness of cancer 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)?

There is an urgent need to develop novel strategies to combat cancer, in particular to combat the spread of cancer and the development of treatment resistance. This project will provide new knowledge about the mechanisms of cancer metastasis and are designed to identify ways by which we can (a) block the interaction of cancer cells with their microenvironment, (b) enhance the effectiveness of cancer therapies.

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

~19000 mice comprising both genetically altered and wild type over a 5 year time period.

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?

Mice will be housed in cages with sterile bedding, food and water. Trained competent personal with experience of using mice in cancer research and who are familiar with the effects of anti-cancer drugs on rodents will perform all procedures. Studies will be designed to use the minimum number of mice. The welfare of mice entering a study is closely monitored throughout each procedure. We will use timely remedies to prevent or reduce the extend of unnecessary pain, suffering, distress or lasting harm. Anaesthesia and analgesia will be used to minimise stress and suffering. The procedures chosen are always considered to be the least severe ones that would produce satisfactory results. At the end of an experiment, mice will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The spread of cancer cells from the primary tumour to distant sites such as the lungs, brain and bones is a multistep process involving (a) cancer cell invasion and remodelling of the surrounding tissue, (b) recruitment of new blood vessels, (c) escape into the circulation and transport to distant sites, and (d) the ability to productively colonise these secondary sites. Substantial amounts of information have been obtained by studying and manipulating cancer cells in the laboratory and such studies continue to be the major part of our activities. However, cancer cells cultured in the laboratory are very different from when they are in the body and there are no good culture systems to model the complex architecture of the tumour and the interaction of the cancer cells with the normal tissues of the body. Most importantly, culture systems cannot be used to adequately study the response of the tumour to drugs. For this reason we need to study these complex cellular events in a mammalian system (mouse) system.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We make every effort to ensure that the minimum number of mice will be used. We will (a) use non-invasive imaging so that each mouse can be followed throughout the course of an experiment without having to sacrifice mice at specific time points, (b) involve statistical experts to ensure that we are using the optimal number of mice in

each experiment, and (c) take great care to ensure that each experiment is analysed in depth (often by different researchers in the lab working on different parts of the project and/or by working in collaboration with our colleagues) and that the maximum amount of information is gathered. This avoids unnecessary experimental repeats.

Refinement

Explain the choice of animals 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.

Refinement

Only mice will be used in this project. The models that we will be using to study primary and metastatic tumours are routine and refined procedures that we have experience with in our laboratory and are in regular use in the cancer research community.

We will make every effort to ensure the optimal welfare of the mice by (a) adhering to the national guidelines for the use of mice in cancer research, (b) using non-invasive methods to monitor tumour growth and response to therapy, (c) only having experienced staff undertake the studies.

PROJECT 80

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Dissecting PRRSV-neutralising antibody responses
Key Words	Pig, Porcine reproductive and respiratory syndrome virus, Neutralising antibodies, Vaccines
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The porcine reproductive and respiratory syndrome virus (PRRSV) is responsible for the most economically important disease affecting the global pig industry. The rapid evolution of PRRSV poses a major challenge to effective disease control since available vaccines show limited efficacy against divergent strains. Knowledge of the targets of virus-neutralising antibodies that confer protection against diverse PRRSV strains would be a catalyst for the development of next-generation vaccines. Key to discovering these new targets are the isolation and characterisation of virus-neutralising monoclonal antibodies from immune pigs. This project will utilise two complimentary cutting-edge technologies to isolate and characterise naturally occurring porcine monoclonal antibodies capable of broadly neutralising PRRSV.

We will identify the antigens and epitopes these antibodies recognise and explore the use of artificial cell membranes embedded with PRRSV envelope proteins as means to deliver these immunogens.

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 proposed project will characterise the neutralising antibody response to PRRSV and isolate porcine monoclonal antibodies with broadly neutralising properties. The isolation of these virus neutralising monoclonal antibodies will enable studies to identify highly conserved epitopes which may be exploited for the development of safe and efficacious PRRS vaccines. By opening a new avenue to improved PRRS vaccines this project should ultimately result in enhanced PRRS control and improved animal welfare and productivity in the pork industry. The establishment of tools and techniques to study antibody responses in pigs will be an important new

resource for researchers to use to study responses to other important pathogens of swine such as porcine epidemic diarrhoea, foot-and-mouth disease and influenza A viruses.

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

148 pigs (Sus scrofa) and 96 mice (Mus musculus) over 5 years

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

Mild to moderate clinical signs for a few days duration following primary inoculation with PRRSV and subsequent challenge infection with more virulent heterologous strains. All animals will be clinically monitored post-infection. Assessments and interventions as appropriate will be performed at predefined frequencies in the experimental protocol, including euthanasia on welfare grounds if required. Parenteral immunisation of animals with adjuvanted ACM vaccines may induce transient localised inflammation. All animals will be euthanized at the end of the experiments.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The biological complexity of the immune system means that there is no alternative to the use of animals to study the neutralising antibody response to virus infection. Alternatives to animal procedures will be used in the proposed project where possible, e.g. cultivation of virus and evaluation of antibody characteristics e.g. virus-neutralising properties in cell cultures.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The animal studies are designed to maximise collection of biological materials/data from each study, and enhance the development and use of *in vitro* and *ex vivo* methods where appropriate. The studies are designed to ensure the appropriate number of animals are used - numbers are selected that enable robust experimental design compatible with obtaining reliable and meaningful results. Pilot experiments

with small numbers of animal numbers will inform and refine the experimental design of subsequent comparable studies.

Refinement

Explain the choice of animals 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.

Refinement

The only species known to be susceptible to infection with PRRS virus are pigs and wild boar. Consequently, no alternative to the use of pigs has been identified. In addition, as pathogen-specific immune responses vary considerably between susceptible and non-susceptible species,

it would not be appropriate to conduct a detailed evaluation of neutralising antibody responses to PRRS virus in a non-susceptible less-sentient host species.

General measures taken to minimise welfare costs to animals include: where possible the use of low virulence PRRSV strains that will not cause overt disease, a rigorous clinical scoring system to monitor animal welfare and trigger humane end points, reducing sampling frequency and volumes to a minimum and to reduce stress by positive reinforcement with rewards following sampling.

PROJECT 81

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Lymphocyte trafficking in health and disease.
Key Words	immunology
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Lymphocytes, the cells of the immune systems which specialize in effector responses, such as killing of virus-infected cells or transplant rejection, are activated in the lymph nodes by other cells, called antigen-presenting cells. After activation, they must migrate to the site where the immune response is taking place (i.e. the site of infection or the transplant) to carry out their function. The mechanisms that drive activated lymphocyte migration to antigen-rich organs are not well understtod, however it is known that in chronic inflammatory diseases and cancer these mechanism are altered so that the immune response does not resolve (myocarditis, chronic heart failure) or it is not efficacious (cancer).

The aim of this project is to understand the basic mechanisms of lymphocyte migration and how these are altered during disease. Importantly, we aim to develop strategies to correct lymphocyte trafficking during disease as novel therapeutic tools for human diseases and test these strategies in relevant models of disease to favor their fast progression to clinical use.

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

The primary potential benefit of this project relates to new knowledge about regulation of lymphocyte migration to specific parts of the body. The aim is to publish the findings in academic journals. The information will be of interest to pre-clinical immunologists. The secondary key benefit relates to the value of the results to clinicians, particularly in the field of immunotherapy, and to the possibility that new molecular targets may be identified, for which new drugs could be developed for a variety of human diseases in which inflammation plays a major part, from cancer to transplant rejection. These treatments will be able to be applied to patients after further pre-clinical studies. We believe that these are likely to improve survival and

quality of life of a large number of patients. Treatment cost for these patients may also be reduced.

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

A total of approximately 300 mice/year will be used by each member of my group to complete this project. This relatively large number is due to the inclusion of genetically altered animals, which are extremely useful to clarify the role of specific cells or molecules in the regulation of lymphocyte trafficking. These mice need to be bred for several generation before they are suitable for experimentation so the majority of them (70%) will not undergo any procedure. All possible efforts to reduce, refine, replace the animal use have been and will be made as stated in the 3Rs section below.

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

The models have been chosen based on their relevance to human diseases and most involve a mild/moderate discomfort for the animals. Only 2 of the proposed procedures, which will account for 10% of the experiments, have substantial severity but we have refined our techniques to develop the least invasive models and minimise death and suffering of animals. Of note, as part of the project, most animals involved in the severe procedures will receive treatments to ameliorate/cure disease. At the end of the protocol, animals will be humanly killed and tissues and organs will be collected for further examinations, in order to maximise the amount of information that we can achieve from each experiment and reduce the number of animals used in the project.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The plan is to identify key molecules that regulate lymphocyte migration to specific sites of the body. This involves a lot of in-vitro laboratory work, using tissue culture techniques. Migration of lymphocytes is an extremely complex project which involves the lymphocyte to localise to different site of the body by interacting with other cells and crossing complex barriers (vessel walls). Full testing requires a fully formed vascular network, which is not achievable by using computer-based systems, lower organisms and embryo stages, cultured cells, tissue, and organs. Only living animals can be meaningful models for the purpose.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

A wide literature search has confirmed that our project is original, and that there is no duplication with previous reports. For quantitative experiments, animal numbers needed are statistically determined using power analysis. We have refined suitable models over the years, which allow to use a small number of animals to achieve statistical significance.To assure reproducible outcome, which will maximises the information obtained from the minimum resource, experiments will be carefully designed and performed, including randomisation of treatment or control groups, allocation concealment, and blinded assessment of outcome, and explicit inclusion and exclusion criteria. In addition, tissues from the same animal will be used in as many analyses as possible to minimise the number of animals required.

Refinement

Explain the choice of animals 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.

Refinement

We will use mice, which are the most suitable for this large-scale basic and translational study. General features of lymphocyte trafficking in murine models are sufficiently equivalent to humans. In addition, we will be able to use genetically altered animals, an extremely useful tool to elucidate basic and pathologic mechanisms while reducing procedure on animals (e.g. continuous administration of inhibitors erc). There are a plenty of useful research materials (like antibodies) for mice.

We will use a variety models that mimic either physiological or pathological lymphocyte migration. All the models are well justified and have been widely used in the similar research.

Three of the models proposed have “substantial severity”, but we have optimised the protocols to mininise the death and distress of the animals. Surgical and anaesthetic techniques have been refined. Post operatively, animals will receive intensive care for several hours in special recovery cages. Post-operative pain will be prevented using analgesics. Infection will be prevented by using antibiotics and aseptic procedures in a specifically-regulated recovery surgery room. Dehydration will be prevented by administration of fluids and limited blood collection. In an unlikely event in which animals do not recover from surgery well or develop unexpectedly severe heart failure, they will be humanely killed.

PROJECT 82

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Lysosomal dysfunction in human disease
Key Words	Lysosome, lysosomal storage diseases, glycosphingolipids, rare diseases, neurodegeneration
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Lysosomal storage disorders are a group of over 70 devastating inherited human diseases that most commonly affect infants, children and young adults. They occur at a combined frequency of 1:5000 live births and are the commonest cause of neurodegeneration in children. The brain is often affected and symptoms including dementia, loss of coordination, loss of speech, visual system problems, mental retardation and seizures. There are currently no effective treatments available and so these children die prematurely.

We have two main research aims. 1) To better understand how storage of molecules in the cells recycling centre (the lysosome) causes such devastating disease and 2) to use this knowledge to develop therapies for patients. We will also help our collaborators to characterise newly discovered diseases that may have similarities with the lysosomal diseases that we study.

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 benefits of the project are to gain a better understanding of disease pathogenesis and in so doing identify novel clinical intervention points. [REDACTED]

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

Mouse Approximately 37,500 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 levels of severity? What will happen to the animals at the end?

Typical symptoms include ataxia, weight loss, reduced activity and muscle weakness. Under the breeding programme of heterozygotes for Sandhoff and NPC mice approximately one quarter of live births will be homozygotes for the mutation. These mice have well-defined disease progression very similar to patients including ataxia, hind limb stiffness, loss of vision, tremor and inability to interact socially. Well-established humane end points have been established and mice will be killed under Schedule 1 or by perfusion under terminal anaesthesia (AC). Under experimental procedures studying new disease models and trialling therapies mice will go to late humane endpoints. This is to determine longevity and behavioural function of the model over their life span with and without a therapeutic intervention to better predict outcomes in patients. Animals will be schedule 1 killed and typically analysed biochemically and using histopathological evaluation.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Tissue culture models are used where appropriate e.g. patient cell lines (fibroblasts and lymphoblasts) to investigate cellular pathology and to see if therapies can correct the disease at the level of the single cell. However, there is a clear need to study intact body systems such as the brain and immune system which makes animal usage essential. Studies of behaviour and function [objective 2 and 3] are not currently possible in vitro as these culture-based systems do not replicate the complex in vivo functions of the brain and other organs/systems. Although the screening of therapeutic compounds is performed initially in vitro, it is still necessary to determine whether these treatments have any therapeutic benefit in intact living animals (in this licence authentic mouse models of these diseases) prior to translational studies in patients, as this is a regulatory/clinical requirement.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

A minimum colony size will be maintained for each mouse model, following the advice of our NACWO. Data will be subject to statistical analysis using an appropriate statistical methodology. Our previous experience, and that of other investigators conducting research in this area, indicates that we usually need to perform each experiment on 5-10 animals to provide sufficient confidence levels for appropriate calculation and interpretation; a statistical significance of 5 % will be used throughout. Where practical we will use computer-generated randomisation

protocols to randomise experimental groups. In addition, where practical analysis of data will be performed blinded, to minimise bias introduced by the investigator. We will carefully consider minimal group sizes, number of groups to be studied, use of one or both sexes of animals as appropriate, optimising protocols and sharing of organs and tissues etc. from all our studies (common practice in this laboratory). Small-scale pilot studies are always conducted to see if larger scale studies are warranted. In addition, our ability to use pharmacological induction of LSDs in a broad range of available inbred mouse strains will allow us to identify potential genetic modifiers using bioinformatics approaches without the need to make crosses of GM LSD models onto other background strains providing substantial reduction in animal usage. It is not possible to predict the genetic background that will modify LSD phenotypes and will require experimental determination.

Refinement

Explain the choice of animals 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.

Refinement

Large animal models of LSDs exist (e.g. cat, dog, sheep etc.) and we collaborate with veterinary groups that maintain them to confirm findings we make in the mouse models, prior to clinical studies. However, the mutant mice used in this project represent the species of the lowest neurological sensitivity to which the protocols can be successfully applied and which are readily amenable to genetic manipulation and crossing with other GM and non-GM strains.

[REDACTED]

PROJECT 83

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanisms of Neuronal Function and Dysfunction
Key Words	synapse, ion channel, hearing, deafness, brain, intrinsic plasticity, cognition
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We are yet to understand how the brain manages to process all the information it receives from our senses. The aim of this project is to explore how recent sensory experience (in hearing) changes the way the brain perceives sound. For example, when you come out of a loud music concert, your ears are ‘ringing’ (tinnitus) and your ability to understand conversations in noisy places (i.e. a bar) is compromised for a few hours. We are also investigating how the basic molecular machine is built up from experssed proteins to a living neuron, how these are assembled into interacting networks, and how they achieve specific functions such as 'sound location', 'gap-detection', 'extraction of a signal from a noisy environment', and how such building blocks form concepts and cognition. There are important implications fo this knowledge to understand mechanisms of disease; for example, loud sounds also cause hearing loss and similar kinds of damage underlie a stroke or in epilepsy. We are developing animal models of human diseases by editing the human mutation into the mouse so that we can better understand hearing loss, ageing and dementia.

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

If we understand the way the brains ‘listens’ to sound, then one benefit is to improve treatments for hearing loss and deafness. The study of synaptic transmission provides fundamental knowledge of brain function which benefit and improve treatments for stroke, epilepsy and neurodegeneration. We will make animal models of human neurodegenerative disease and use them to explore mechanisms of cognition and memory. This basic research will provide fundamental knowledge which will contribute to treatments for neurological disease.

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

These experiments will be conducted on mice, and some rats. They hear well and can be genetically manipulated. Development of a genetically manipulated mouse strain for a human disease requires considerable breeding which over the five year period amounts to around 8000 animals. Around 2000 of these animals will be killed to provide tissue for in vitro experiments. Around 100 mice per year will be used for in vivo experiments to study hearing and cognition in which the animal is trained to perform a task and brain activity measured during that performance. These studies would typically take place daily for 30 minutes to 1 hour and over a duration of 6 months.

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 animals will be humanely killed to provide tissue for in vitro studies with mild severity. Some experiment require exposure to loud sounds (under anaesthetic) which render the animal partially deaf, giving a moderate level of severity. Around 20 mice per year will receive a surgical head implant so as to permit recording of brain activity after they have recovered from the anaesthetic and to study cognition (i.e. mechanisms of thought). Recovery from the surgery takes around 3 days and the animals can freely move with the head implant. During an experiment lasting around 30 minutes, the implant is secured to the recording apparatus, so the animal's head is immobilised, but the animal can run and move the rest of its body, simultaneously. Cognition cannot be studied when the animal is asleep with an anaesthetic. To encourage the mice to learn, the mice have a restricted amount of water (or food, but not water and food) each day and must earn the additional water by performing a task. The task is a simple visual maze, where they must find a virtual 'hole' by moving to their left or right. In this way we can monitor their brain function while they undertake the task. These mice are particularly carefully monitored and the treatment is of moderate severity; if they cannot learn the task they are removed from the experiment. These experiments provide important insights into cognition and memory. At the end of the experiment the animal is humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The brain is the most complex form of matter known. Computational models help data interpretation and analysis, but cannot yet replace the study of a real brain, since a computer programme can only include known parameters, and there are

many unknowns which are yet to be discovered. Investigation of brain function, hearing and cognition need to be studied on a mammal (such as a mouse) since their brain mechanisms are similar to other mammals and humans. We can introduce human gene mutations into a mouse which powerfully aids disease investigation. Non-protected animals such as insects or molluscs have nervous systems which are constructed in ways that make it more difficult to translate and understand human disease.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We attempt to minimise animal use in many ways: by sharing tissue from one animal between multiple in vitro experiments. By working closely with an *in vivo* behaving animal and taking care of its' health and wellbeing we can achieve high quality and high volumes of data from small numbers of animals, consistent with ethical and statistical validation. We plan breeding to fit need (with the limitation that litters vary in size and that animals of a specific sex, age or genotype may be required for a specific experiment).

Refinement

Explain the choice of animals 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.

Refinement

Animal welfare is very important for ethical and for scientific reasons, because collecting data from a stressed or sick animal gives poor quality results. Although there are huge differences in size between a mouse and human brain, the basic mechanisms by which a mouse brain works are remarkably similar to those of humans. In addition, human mutations underlying disease can be edited into a mouse, so generating specific models of human disease. This means we can avoid using mammals such as cats, dogs or primates. There are of course differences between humans, mice and other species, so we must carefully check results and compare our data with published data on humans and other species. Surgical and potentially painful procedures (such as exposure to loud sounds) are conducted under anaesthesia; the signs of pain and infection are monitored and treated. Animals are frequently checked for signs of suffering or discomfort; for example, by their behaviour and their food and water intake. Procedures are continually refined and the advice of specialist animal carers and veterinary surgeons is sort to improve welfare.

PROJECT 84

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Understanding haematological malignancies to improve treatment outcomes
Key Words	Haematological, Xenografts, Therapies, Lymphoma, Leukaemia
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall aims of the project is to better understand the development and progression of blood cancer and to accelerate the entry of new and effective treatments to the clinic.

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

This project will enable us to better understand the normal functioning of blood cells, how blood cancers develop and to find additional targets for the development of new medicines. A persistent lack of models for some of the rarer blood cancers has impaired the development of novel medicines and contributes to a limited suitable treatment options for patients. We will develop novel and better animal models to improve our understanding of blood cancer and to improve the effectiveness of new medicines. These medicines will then be used in more effective clinical trial settings which take into account individual differences in genetics and cancer characteristics. [REDACTED]

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

Mice, 8200 over 5 years

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

Animals produced under this project will develop human-like blood cancers, will receive medicines that aim to improve the treatment of disease and reduce the unpleasant side effects of current cancer treatments (chemo and radio-therapy). We

expect that the severity will be moderate as animals are likely to suffer moderate impairment of their well-being. Some animals will undergo surgery under general anaesthesia as part of the procedures. Adverse events will be controlled by analgesic. Any animals that show significant impairment of normal behaviour or signs of distress will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Our research routinely uses patient primary samples and established cell lines. However, it is difficult to fully recapitulate the physiological function of tissues and of the microenvironment in vitro. The immune system and by extension blood cancers involve at least three different organs in the body (bone marrow, blood and secondary lymphoid tissues such as lymph nodes and spleen), different blood cell types that have tight relationships between them (B-cells and T-cells permanently “communicate” between them to mount an efficient immune response against antigens or tumoral cells) and involved hundreds of different signals (for example cytokines, chemokines, hormones). Due to this complexity the use of single cell types for examining gene function is limited and it is therefore essential to work in whole body systems to provide the precise roles of such genes in the initiation and progression of blood cancers.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals will be minimised by careful experimental design and appropriate statistical analysis.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the organism of choice because they are well studied, can be genetically modified, their immune functions are similar to humans, and many reagents exist permitting detailed experimental analyses which are recognized internationally.

The injection volumes used will be as small as possible as should the sizes of needles. Sampling will require small amount of blood as the downstream in vitro measurement experiment is highly effective.

We have developed a comprehensive distress scoring sheet and health check protocols, which allow us to identify any early signs of disease progression and terminate the procedure to minimise animal suffering.

PROJECT 85

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Discovery of new treatments for pain and inflammation
Key Words	Inflammation, Neuropathic pain, Drug discovery, Therapeutic agents, Analgesia
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aim of this project is to identify and characterise potential new candidate drugs for clinical testing in human inflammatory diseases (such as rheumatoid arthritis) and chronic pain (such as pain from damaged nerves, known as neuropathy). Existing medicines for the treatment of these human diseases have drawbacks, often having serious unwanted or unpleasant side-effects (such as sedation, cardiovascular complications or damage to the digestive system) and new therapeutic targets are required.

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 ultimate benefit of this project is that compounds could go forward for pre-clinical development and ultimately clinical testing in humans. These will form the basis for new treatments for chronic inflammation and neuropathic pain.

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

We will only use rodents for these studies, predominantly rats. On occasions, we will need to use mice when this species is more appropriate because of the activity of the compounds being more comparable to the expected activity in humans. We plan to use a maximum of 11000 rats and 2400 mice during the 5 years course of this project.

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

The experiments are designed and conducted by highly trained personnel to ensure that animals suffer the minimum amount of distress whilst meeting the scientific objectives of the study. In most cases the endpoints of the experiment will be measurements acquired from behavioural tests which are considered minimally traumatic to the animals and are of short duration. Throughout the tests the animals will have full escape routes from the experiment and will be well monitored for signs of discomfort/distress.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Pain is a multi-cellular process involving the peripheral sensory nervous system, the spinal cord and higher brain areas. Pain generation and transmission involves numerous cell types, including: neurons, glial cells, inflammatory cells and other supporting cell types. Analgesic medicines may act at any point in this network; hence it is currently not practicable to model this process *in vitro*. Realistic measures of success such as pain scores, as measured by such systems as von Frey hairs, and inflammation scores (plethysmography) are not possible in, or transposable to, *in vitro* systems and there are no reliable biomarkers for analgesia. The analgesic or anti-inflammatory effects of compounds must be measured in a mammalian model system. Currently, there are no alternatives to this process, although we constantly review the possible alternatives to the use of animals. A variety of *in vitro* tests (such as target activity, selectivity, measurements of permeability etc) are applied prior to any *in vivo* procedure in order to filter the most appropriate compounds.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Animal numbers have been optimised through statistical power calculations to ensure that the results are meaningful and allow to make decisions moving the compounds forward with the minimum possible number of animals. Through the use of the screening cascade applied to the project priority will be given to animal procedures that eliminate the maximum number of compounds at the earliest stage of the screening process. Subsequent efficacy experiments will be conducted on

single compounds at multiple doses, usually 3, compared *vs* a vehicle control and potentially tested alongside a positive control. A decision on whether a compound is efficacious will be made on the basis of achieving statistical significance *vs* the vehicle group. All studies will be done under the strictest experimental design: randomised, blind to the treatment, including positive and negative controls and with the appropriate statistical power.

Refinement

Explain the choice of animals 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.

Refinement

Rodent models, especially rats, are the lowest species that is likely to produce reliable results that may be predictive for human disease. Rats have a well-documented pharmacology, and the utility and the predictive nature of this model organism has been validated repeatedly through the successful development of a large number of human drugs. Rats respond in a reliable and predictable manner to behavioural tests, allowing group sizes to be lower when compared with other animals, minimising animal suffering. The procedures described in this project (such as inflamed paw using Complete Freund Adjuvant or neuropathic model such as Chronic Constriction Injury) are restricted to one paw and aimed at minimising any potential animal suffering. The testing methods to be used to assess hypersensitivity do not elicit acute pain and are short in duration, minimising any suffering. Throughout the tests the animals will have full escape routes from the experiment and will be well monitored for signs of discomfort or distress. During the tests the novel compounds will provide analgesia and during the surgery marketed analgesics will be administered.

PROJECT 86

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Modulation of pathology and repair in autoimmune and degenerative conditions
Key Words	Damage, Repair, Bone, Arthritis, Neuropathy
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aim of our work is to understand what initiates and perpetuates autoimmune and degenerative diseases of the skeletal and nervous system. We are also focussed on how we can stop disease and initiate repair in damaged tissue or organs. The reason we are doing this is because even with all of the work that has been done to date, we still do not understand what triggers and drives disease forward. For instances, what is the impact of obesity on disease and can exercise help. We also don’t know how to repair the body and reduce disability in those individuals that have luckily managed to recover from an episode of disease. To do this we will use a range of experimental approaches to investigate disease pathogenesis and repair in autoimmune and/or degenerative diseases of the skeletal or nervous system

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 new insights into how the systems of our body (immune system or connective tissue cell system) can be regulated. It will provide us with an understanding of the pathways that contribute to the perpetuation of disease and repair processes, which are associated with autoimmune and degenerative diseases. The development of novel intervention strategies (molecular, cellular or physical) requires an advanced understanding of the roles and mechanisms of action of cells and proteins that are dysregulated. This work will provide that. The important long-term benefit of this work is that these studies will identify and validate new approached that can be used to treat or manage these conditions and alleviate the burden of disease and disability for both humans and animals.

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

These studies will use both mice and rats. Over the 5-year period due to the complex nature of the studies we will breed approximately 5000 mice and perform experimental studies on 11,650 mice and 2000 rats. It should be appreciated that the experimental studies will use mice generated in our breeding programme.

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?

In some studies, we will induce arthritis (either rheumatoid or osteo). The rheumatoid arthritis we induce is considered a model of substantial severity whilst the osteoarthritis is considered moderate. In other studies, we will induce the animal equivalent of multiple sclerosis, which is considered a model of substantial severity. During and at the end of the models the animals will be assessed for disease parameters and culled. Once culled appropriate tissue/organs/biological fluids will be harvested and extensively used in laboratory experiments.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The reason animals have to be used in these experiments is due to the multidimensional nature of autoimmune and degenerative diseases. It is not just the immune and/or connective tissue cells that contribute to these interactions but the complex tissue associated microenvironment. Furthermore, ageing, obesity and exercise can also have a substantial impact on these systems. It is, therefore, not possible to duplicate these interactions in anything other than that of an intact mammalian system.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To assure the minimum numbers of animals are used we will only use sufficient numbers necessary for valid statistical analysis, and conduct non-invasive procedure to capture data in the same animal over various time points. Furthermore, we will perform as many experiments as possible on tissues and organs collected from animals under-going licenced procedures. Finally, we will use the information from

the animal experiments to generate and use human *in vitro* culture systems to investigate the human scenario.

Refinement

Explain the choice of animals 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.

Refinement

The disease models to be used in mice and rats emulate the diseases in a way that we are unable to accomplish *in vitro*. The components observed in the human diseases are present in the mouse and rat models and will allow us to validate the efficacy of therapeutic regimes.

Animal suffering will be minimised based on close monitoring, analgesia where appropriate and non-chemical methods such as increased bedding, keeping warm and easy access to food and water.

We have extensive experience in the protocols that are of substantial severity and have defined clear end-points and a robust monitoring system.

PROJECT 87

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Development of novel cancer therapeutic agents
Key Words	cancer, treatment
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

More than 1 in 3 of us will develop cancer during our lifetime. During the last thirty years there have been significant improvements in the diagnosis and clinical management of this disease. However, clearly there is still an unmet clinical need in many cancer types and an urgent requirement for novel treatment strategies. It is imperative to determine the pharmaceutical properties of agents prior to evaluation in the clinic. The principle aims of the project are to understand how much of the therapeutic agent reaches the tumour and whether this elicits an appropriate effect. Ultimately it will be confirmed whether the elicited response results in a consequential therapeutic effect, for example a reduction in tumour size.

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)?

Many of us are likely to develop cancer during our lifetimes. Current chemotherapeutic agents are associated with non-selective side effects and tumours can develop resistance to the agent resulting in the death of cancer patients from disseminated cancer. Consequently there is an urgent medical need to develop targeted therapies for each specific type of cancer. Pivotal to this development is the translation of basic oncology laboratory findings to agents with few side effects with good pharmacological properties and potential for clinical development. This project licence will facilitate in the building of a preclinical package to support clinical development of a clients' substances. The ultimate long-term benefit of this work, identification of a clinical candidate for the treatment of a specific cancer, is beyond the time-frame of this licence albeit critically underpinned by this work. However, there are key short and medium term milestones that will be achieved. Firstly, short-term milestones on each project, will include optimisation of the pharmacokinetics of the inital compound received from the client. This will enable adequate levels of the agent to reach the tumour to elicit a pharmacological response. Treatment of cancer

is frequently associated with toxicity. The second key milestone, will be identification of compounds that can elicit a pharmacological response at well tolerated doses. Both of these two key milestones will be go/no go decision points for the project, enabling the optimal compounds to progress to be tested in proof of concept studies and achievement of a medium term milestone, i.e. to confirm that modulation of the target leads to a therapeutic response. Importantly, these agents may also target secondary tumours that have metastasised from the primary tumour, which represents and an unmet clinical need. Each of these three critical pieces of data will enable a project to progress to late pre-clinical development/clinical development or of equal importance for the project to be closed, in order to prevent sub-optimal agents being evaluated clinically and the waste of resources.

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

The mouse is well validated as a model for the development of cancer therapeutic agents. Immunocompromised and immunocompetent mice will be used in the case of human and mouse tumours respectively. It is envisaged that 10,000 will be used during the 5 yrs of the project licence.

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

The mouse is well validated and accepted by the scientific community as the model of choice for the evaluation of efficacy of cancer chemotherapeutic agents. We will carefully determine the dose required to elicit the biological effect, whilst causing the minimum suffering to the animal. We use this dose to design the efficacy study in mice bearing tumours derived from human cancer cells. The tumours are normally grown in the flank of the mouse and do not impede movement. Large tumours can affect movement, but in this project tumours will not be allowed to grow beyond a pre-determined maximum size. Mice will be monitored carefully for any distress caused by the tumour, although such distress is not generally observed. Where internal tumours are required for example in the peritoneum (which is more reflective model of ovarian cancer in humans) increased frequency of monitoring will be needed to facilitate recognition of abdominal pain and/or loss of body condition. In the case of models of metastasis additional clinical signs include ruffled fur, rapid breathing and hunched posture, which will be carefully monitored for. Following administration of the agent to the mouse, the animal will be carefully monitored for adverse effects, however these should already be understood and minimised based on the information derived from the dose finding studies. Agents may cause toxicity, as reflected by weight loss, but this will be carefully mitigated by escalating doses slowly and only after the initial dose has been confirmed as being tolerated. Other signs of toxicity may also be observed, for example hunched posture, inactivity and lack of interest in food. Routes of administration will typically include oral administration and intra-venous injection, which cause only momentary discomfort.

Similarly other injection routes, subcutaneous, intramuscular and intraperitoneal, used occasionally, only cause transitory pain. Blood may be sampled from the tail vein, causing transitory discomfort. In some studies, general anaesthesia will be used, for example to enable in life imaging of the tumour. Furthermore, in the case of breast cancers that require oestrogen for growth, pellets that release these hormones will need to be implanted under the skin. At experimental cessation, mice will be humanely terminated.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Whilst in vitro systems provide us with important data on the properties of novel agents, it is imperative for these properties to be determined in the intact biological system, e.g. the mouse prior to progression towards the clinic. Only lead compounds with optimised properties are evaluated in animals. Numerous assays will be used to select optimal compounds. Consequently, only agents that inhibit the target and kill cancer cells and have suitable properties will progress to tests in animals. There are a number of new systems that allow efficacy to be measured in vitro and these will be used wherever possible. However, if these in vitro tests alone were used to select agents for evaluation in humans, there would be two consequences. Firstly, compounds wth sub-optimal properties would be administered to sick cancer patients, which ethically would be unacceptable and secondly, potentially toxic agents would be given to patients already unwell as a consequence of their tumours.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use the minimum number of animals necessary to give statistically meaningful results. This is based on extensive previous experience of completing animal studies of this nature, and is continuously reviewed in the light of new tumour models which may behave in a more predictable manner and could therefore lead to a reduction in the number of animals used. We will use existing information to inform our choice of tumour models and dosing regimes. A minimum group size will be used (typically 8-10) based on a statistical analysis of the experimental variation attained during the model development stage (with statistician advice sought where required). Mice will be typically randomised prior to treatment based on tumour size and assigned to treatment groups.Furthermore, as we have experience of optimising a substantial number of models, we are confident in their reproducibility and are

therefore able to use the minimum number of animals. Wherever needed statistical advice will be sought and where possible experimentalists will be blinded to the identity of experimental groups to avoid bias.

Refinement

Explain the choice of animals 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.

Refinement

The mouse is used for evaluation of new cancer chemotherapeutic agents as it is well validated both in the scientific literature and by the pharmaceutical industry. Tumours derived from cell lines can be readily grown in immunocompromised mice and this provides a means of demonstrating proof of concept of the agents of interest. Mice are well establshed for such procedures, relative to a simpler organisim such as the fish, due to the intact immune system (for immuncompetent mice), circulatory system and presence of mammalian organs.

Mice will be group housed wherever possible and environmental enrichement provided. In terms of welfare the least invasive model will be used in the first instance. Similarly, for administration of therapeutic agents, the least invasive route, e.g oral, will be used where possible.

When designing studies the Guidelines for the welfare and use of animals in cancer research by Workman et al., 2010 will be used. Mice will be monitored daily for any adverse events resulting from treatment, with comparison with age and sex matched controls. Appropriate analgeisa and anaesthesia will be used for surgical procedures.

PROJECT 88

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Cellular mechanisms underlying auditory defects
Key Words	Hearing Loss, Tinnitus, Cochlea, Brain, Plasticity
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Hearing loss is the most common sensory disability, affecting nearly 20 % of the population and tinnitus (“ringing in the ears”) is often, although not always, associated with hearing loss and already affects about 5 million people in the UK. Unlike hearing loss, for which treatment is available with hearing devices, there are no effective treatments available for tinnitus sufferers, and no licensed pharmaceuticals for this indication. The aim of the project is to determine which molecular mechanisms are dysregulated after exposure to loud sound leading to hearing loss and / or tinnitus and to develop novel therapeutic strategies for the treatment of hearing loss and/or tinnitus. The project uses rats and mice which responses to acoustic trauma are well characterised. It will also use zebrafish as an alternative model for noise-induced hearing loss, and transgenic mice with a view of identifying the genetic underpinnings of hearing loss and tinnitus. Use of MRI imaging will allow pin pointing to brain areas activated or inhibited during hearing loss and/or tinnitus.

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 project will allow understanding mechanisms responsible for hearing loss and tinnitus and is therefore likely to bring a very significant healthcare benefit to a wide population suffering from those auditory deficits. It will also allow testing novel therapeutics such channel modulators and potentially give raise to efficient compounds targeting hearing loss and tinnitus.

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

We expect using 1000 rats, 1300 mice and 100 zebrafish 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 levels of severity? What will happen to the animals at the end?

The level of severity is anticipated as moderate due the exposure to loud sound that induces hearing loss and tinnitus. This could potentially increase animals 'stress level. Stress level can also be increase due to repeated anaesthesia. We have never observed signs of stress (such has reduced appetite or piloerection or impaired behavioural activities such as licking or lethargy) during or after acoustic over-exposure, or following repeated anaesthesia. Should this happen, animals would be humanely killed. At the end of study, animals will be transferred under another authority, or will be humanely killed for tissue sampling and in vitro studies. Animals will be killed either by decapitation or by schedule 1 method, at different time periods following exposure to loud sound (when their hearing threshold is elevated and/or or when they experience tinnitus). Genetically altered animals produced under this protocol are not expected to exhibit any harmful phenotype.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animal models are used here to allow understanding mechanisms responsible for hearing and tinnitus, and testing for potential therapeutics. Animals used in this current studies consist principally of rats and mice which genomes of very similar. We will be doing in vitro recordings on slices alongside in vitro characterisation of specific morphological markers. We will also be doing computational studies to complement our study and use the zebrafish (Danio rerio) which represents an ideal alternative vertebrate model of human pathologies because of its high conservation of genetic information.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The numbers of animals will be minimised by careful experimental design and appropriate statistical analysis. We will also ensure maximising the number of data from in vitro experiments to limit the number of animals.

Refinement

Explain the choice of animals 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.

Refinement

The rodent tinnitus model proposed in this project is a well-established model which is routinely used in the lab.study hearing loss and tinnitus. Rats or mice undergo an exposure to loud sound which is followed by noninvasive auditory brainstem recordings and gap-induced prepulse inhibition of acoustic startle (GPIAS) which is testing for tinnitus. Knockout mice will also be tested to study genetic defects behind hearing loss and tinnitus.Animals are anesthetised during the auditory brainstem recording procedure as movement would impair recordings. They are also anesthetised during the early stage of acoustic over exposure and sedated during the main part of the exposure as our pilot experiments have shown that they need to be conscious for the biological consequences to occur. Sound is progressively increased to its maximum level over a period of 5 minutes. Using this procedure, we never encountered any behavioural signs of distress or discomfort. Some experiments will be performed on zebrafish which could be subsequently used as an alternative model for hearing loss. Non invasive MRI imaging will be used. MRI imaging represents a refinement as this method does not require use of contract agents, and involves only anaesthetics for restraint, well tolerated by animals.

PROJECT 89

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Modulating inflammation in the GI tract
Key Words	Inflammation, Intestine, Experimental therapeutics
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To assess the effectiveness of experimental therapeutics in animal models of inflammatory bowel disease, for the purpose of determining their potential for treating inflammatory bowel disease (IBD) in humans. Methods of investigation will be improved and developed within the philosophy of the 3Rs.

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)?

These data obtained will be used by Sponsors, to determine whether the drugs are suitable for the treatment of human inflammatory bowel disease.

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

Mice 7500. Rats 250.

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

Animals may experience body weight loss and diarrhoea. In some instances, they may pass a small amount of blood in their stool. The expected level of severity is moderate and humane end points will be applied to all protocols in order to maintain this. All animals are euthanised at the end of a study so that changes in the structure and function of the bowel can be analysed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The gastrointestinal tract is perhaps the most complex organ system in the body. In addition to food metabolism, it is a major organ of the immune system and is home to several hundred species of microorganisms. Producing a non-animal model in a lab, which accurately replicates all aspects of gut function and dysfunction, is currently not possible.

We have a range of in vitro laboratory techniques that are able to address some of the individual aspects of gut and immune system function. Such in vitro experiments will have been performed by us or our Sponsors prior to any research on animal models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

All experiments are conducted according to current best practice. Statisticians from academia and from independent companies are consulted to ensure the rigour of our experimental design and analyses. We are promoting multi-user studies to improve both the efficiency of animal usage and the robustness of individual studies.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the least sentient species most suitable for these studies. In some instances, a rat model may be more appropriate. Rodent models of inflammatory bowel disease are well-characterised and demonstrate broad similarities with human disease, in regard to symptoms and pathological changes within the intestine. Also, the models are responsive to agents that are used to treat human IBD. We have refined many aspects of running the models, including the optimisation of dosing protocols for different therapeutic drugs; increasing the number of different data read-outs that can be obtained from a single study; built up an historical data base that allowsmore accurate experimental design. Studies run for the minimum period of time sufficient to achieve their objective(s) and appropriate humane end points are employed.

PROJECT 90

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Electromechanical coupling and diastolic function
Key Words	Heart failure, Diastolic function, Repolarization
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Heart failure is a major unmet clinical need and is associated with a poor patient prognosis. There is good indirect evidence that abnormalities in the hearts electrical activity may contribute to the inability of the failing heart to pump blood correctly, but little work has been done on the mechanisms relating these observations and how they might be targeted therapeutically. This is the primary aim of this project.

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

This project will investigate in detail the relationship between electrical and mechanical dysfunction in the failing heart. It is feasible that these studies may result in the development of a novel therapeutic strategy for the treatment of patients with heart failure.

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

Guinea pigs – 900 over 5 Years (180 per year)

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?

This project will to investigate the relationship electrical and mechanical dysfunction in the failing heart - a complex phenomenon that cannot be adequately studied without animal models. Studies will be conducted primarily in tissues isolated under terminal anaesthesia and with little or no suffering. In a limited number of studies animals will undergo a surgical procedure to induce heart failure, as seen in patients. Animals will be given appropriate care, including pain relief, to limit any pain and suffering. Animals will be continuously monitored for signs of distress and, if

necessary, humanely euthanized. Few adverse events are expected with this approach.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Cardiac electrical and mechanical function is complex, involving the interaction of multiple factors and cannot now be studied without animal models. Our understanding of the processes involved, and their relative importance, limits our ability to use computer modelling, though this is a goal we are working towards.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Experimental data will be continuously analysed and assessed to achieve the aims of the project with the minimum number of animals. All protocols will be refined and conducted by trained individuals, to reduce errors and experiment numbers. Studies will conform to the NC3Rs ARRIVE guidelines.

Refinement

Explain the choice of animals 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.

Refinement

Guinea pigs are the smallest laboratory species relevant for the study of cardiac electrophysiology, being more similar to humans when compared to rats and mice. The guinea pig will provide the most clinically relevant data and any findings are more easily translated to humans.

Our experimental protocols have been developed to limit harm to the animals, being as short as reasonably possible and mainly conducted under general anaesthesia. We will continue to make efforts to refine protocols and further reduce the welfare costs.

PROJECT 91

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Host-pathogen interactions in the immune system
Key Words	intestine, prions, immune system, infection, pathogenesis
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Prions are a unique group of prolonged diseases which cause extensive nerve damage in the brain. In the absence of a cure these diseases are invariably fatal. These diseases affect both animals and humans, and include Creutzfeldt-Jakob disease (CJD) in humans, BSE in cattle, chronic wasting disease in mule deer and elk, and scrapie in sheep and goats. Some animal species and humans have become infected with these diseases after eating food contaminated with prions, or through lesions or cuts to the skin, mucous membranes or cornea. Many questions remain concerning the route the infectious prions take from the site of exposure (eg: intestine) to the brain where they cause damage to nerve cells.

[REDACTED]Therefore, the **first major aim** of this project is to determine how infectious agents such as prions hijack the body's immune system to establish infection. In addition, **the second major aim** is to understand how additional factors such as inflammation and co-infection with other pathogens affect the function of the immune system, and disease susceptibility and pathogenesis.

Key objectives are as follows:

-To enhance our understanding of the development and function of cells and tissues within the immune system.

-The immune system acts to provide protection against infectious diseases. However, some pathogens have evolved to hijack certain immune cells in order to establish infection. This licence aims to determine which cells and tissues within the immune system are exploited by pathogens such as prions and gastrointestinal helminths to infect the host.

-To determine the impact that pathogens can have on the function of the immune system and the effects this has on susceptibility to infection with another infectious diseases.

-Ageing has a dramatic effect on the immune function. Studies in this licence aim to determine the impact of ageing on the function of the immune system and the effects this has on susceptibility to infectious diseases.

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)?

One of the main pathogens that will be studied in this project is prions which affect humans and animals. Infection with prions causes extensive neurodegeneration in brain ultimately leading to death. Prion diseases are currently untreatable. If we can understand the mechanisms that prions use to establish infection, we may be able to design novel therapeutic strategies to block these currently untreatable diseases. Over 26% of the UK population is >65 years old and this is expected to rise significantly in future decades. Immunity in the elderly is significantly compromised by ageing and is associated reduced vaccine efficacy and increased incidence of infectious diseases and cancer. A thorough analysis of the factors that underpin the dramatic ageing-related decline in immune function will help identify the ageing-related factors that influence pathogen susceptibility and aid the development novel approaches to improve immunity in the elderly.

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

In this study it is anticipated that up to 3000 mice may be used over the entire 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 levels of severity? What will happen to the animals at the end?

Some of the mice in this study will be infected with prions and will develop clinical disease: moderate severity. To avoid unnecessary suffering these mice will be culled at the onset of definite clinical signs of prion disease. The duration of oral prion infection in mice is very long ~340 days. However, for approx. 90% of this period the mice are free of clinical signs and appear healthy. At the end of the experiment, many tissues are collected after death from these mice for additional laboratory analyses to study the magnitude and distribution of the prion disease in these animals. Other mice may also undergo periods of moderate severity, for example after irradiation. However, in each of these instances the period of severity is very transient as the mice are given a bone marrow transplant. In the majority of these studies lymphoid tissues and cells will be collected from most mice after death for further study in the laboratory.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The aims of this study are to determine how pathogens such as prions exploit cells and tissues of the immune system to spread from the site of exposure (eg: intestine) and establish host infection. This cannot be achieved using species outside the animal kingdom which do not have complex digestive, immune and nervous systems. However, where possible and appropriate *in vitro* systems will be used as a replacement for the use of mice. For example, recent technolgy has been created which enables mini-guts to be grown in the lab. Although these mini-guts contain only the single cell layer of cells which lines the intestine, they provide an excellent alternative system in which the early interactions of certain pathogens with the gut epithelium can be studied. In recent years we have successfully developed this system to enable us to prepare mini-guts from the intestines of mice, cows and pigs.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Good principles of experimental design will be applied to ensure the minimum numbers of animals are used to achieve robust and reliable results.

All experimental plans have to be critically reviewed by the named Vet, NACWO, NTCO and a statistician before they can be undertaken. A study request is submitted which includes a description of the objectives, hypothesis, experimental groups, treatments, and assessment of effects, methods of data collection and statistical analysis. This review system is intended to ensure that the minimum numbers of animals used in each experiment, whilst ensuring that robust and meaningful data are obtained. No study involving the use of experimental animals can be undertaken without having successfully undergone this critical review.

Refinement

Explain the choice of animals 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.

Refinement

The *in vivo* experiments will use mice. A mammalian species is necessary since the main aim is to investigate interactions of certain pathogens with host immune cells and tissues. Data generated from this project will be directly relevant for enhancing our understanding of disease pathogenesis and susceptibility in large animal species (sheep, cattle, cervids etc.) and humans. Among mammalian model organisms, the information and literature on basic anatomy and physiology of the immune system is most extensive for mice. This species provides the best platform for understanding data produced in this project in the context of previous work. Although differences do clearly exist between species, the major features of the immune system are conserved in mice and humans allowing meaningful extrapolation. Good technique (e.g. aseptic) for each procedure as well as extensive monitoring and defined endpoints will minimise harm.

PROJECT 92

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Epigenetics of cell senescence, cancer and ageing
Key Words	senescence, cancer, ageing, epigenetics
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Chromatin is a composite of DNA, protein and other biological molecules that compacts, packages and organizes the cell's DNA into the cell nucleus. Chromatin organization has a profound impact on cell and tissue function. For example, the difference between a liver cell and a muscle cell is determined entirely at the chromatin level. Likewise, changes to chromatin contribute to cancer and age-associated changes to cell and tissue function, including diseases of ageing. The purpose of this application is to use animal models to address the role of chromatin in aspects of aging and cancer biology that cannot otherwise be addressed in cell culture or alternative models. Specifically, we will investigate the role of chromatin in 1) suppression of cancer; 2) tissue ageing and 3) wound healing (impaired wound healing is a common hallmark of tissue ageing).

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)?

These studies will determine which chromatin regulators are critical determinants of cancer and tissue aging. This will determine whether these regulators represent good targets for biomedical intervention. The models established will provide in vivo models to better understand the function and mechanisms of these chromatin regulators.

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

All studies will use mice. We expect to use approximately 30,000 mice over 5 years.

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

Animals will be bred to show predisposition to specific cancers, such as melanoma or pancreatic cancer, or will receive a transplant of tumour tissue or cells from mouse or human cancer. Approximately 80% of the mice will not show any adverse effects related to the breeding and not undergo any procedures except for ear notching for identification and genetic testing. These 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. Some proportion of the animals (approximately 20%) will be predisposed to cancer and will be monitored carefully for clinical symptoms, weight loss, swelling of the abdomen and development of visible or palpable tumours. Mice with tumours will be monitored carefully by trained staff and if the tumours reach 1.2 cm, or become ulcerated or interfere with normal behaviour, mice will be humanely killed and the tissues will be analysed. In some cases, we will treat animals with experimental chemical compounds and measure the effects on tumour growth or spread. This may involve adding substances to the food or drink or injection of substances. All animals receiving treatments will be monitored closely and any animals that display signs of being unwell, such as ruffling of the coat, reluctance to eat or move, weight loss up to 15% will be humanely killed. At the end of the study, all animals will be euthanized. Some proportion of the animals (approximately 20%) will be predisposed to accelerated ageing or treated with chemicals to promote accelerated ageing in specific tissues. Specific ageing phenotypes include premature hair greying, pancreas, liver or intestinal degeneration, weight loss, hair loss and cataracts. Mice with, suspected of or predisposed to such phenotypes will be closely monitored. Any animals that display signs of general malaise, such as reluctance to eat or move, weight loss up to 15% will be humanely killed. At the end of the study, all animals will be euthanized. Some of the mice (<10%) will be subjected to minor skin wounding to assess the wound healing process. We expect to see variations in the rate and efficiency of wound healing, but since wound healing is a normal physiological process and the wounds are minor there should be no debilitating effects. In the event that wounds fail to heal or become infected, leading to discomfort to the mouse, these mice will be euthanized.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Most of our work is done with cell cultures derived from humans or from tissues taken from animals that have been humanely killed, to minimise the amount of work done with live animals. However, some experiments must necessarily be performed in an animal, as cancer cells encounter various organs and tissues, and most often

kill via their debilitating effects on tissue function. Mouse represents the best model for human cancer available to us, due to the ability to manipulate the DNA and test the effects of loss or alteration of specific genes on cancer progression.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We perform pilot experiments using only a few animals for new studies, before scaling up to the appropriate numbers for a full study. Numbers are calculated based on the experience of other local research groups using the same models, published literature and advice of our in-house statistical experts. We also share animals between experimental groups where possible- e.g. when we need normal animals for controls, we can often obtain these from our breeding colonies where they would normally not be needed in a study. We constantly optimise our breeding strategies to minimise the number of animals needed to achieve the desired genotypes for our studies and we use tumour transplant models where appropriate, which do not require breeding of genetically altered animals and thus use fewer animals in total per study.

Refinement

Explain the choice of animals 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.

Refinement

Mouse genetic models of cancer are widely accepted to be the most closely representative of human cancers. The tumour forms in the correct tissue and spreads via the normal routes and the tumours often progress through the same stages of pre-cancer as in humans. We use state-of-the art genetic models to ensure that the cancer develops in the correct organ/tissues and there are as few side effects as possible due to breeding or treatments. This is done with inducible DNA recombination enzymes that are specific to the target tissues of interest and is achieved by breeding these into the genome. Animals will receive anaesthetic and/or analgesic treatments where appropriate. All animals will be monitored regularly for signs of normal behaviour and will be humanely killed if they exhibit moderate adverse signs.

PROJECT 93

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Breeding, production, archiving and the application of assisted reproductive techniques of genetically altered mice
Key Words	Mouse, Genetically Altered, Cryopreservation, IVF
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This license will permit the creation, archiving and introduction of genetically altered animal models into a world leading academic research institute for the duration of 5 years and will facilitate the efficient and ethical management of each live resource throughout the duration of various research programmes.

Key objectives can be summarised as;

1. The generation of up to 40 novel genetically altered mouse lines per year over the course of the license using appropriate methods via in-house genome editing services.

2. To maintain breeding colonies of genetically altered lines for distribution to various research projects.

3. To provide the application of assisted reproductive techniques for the purposes of efficient colony management and the introduction of new mouse lines into the Biological Services Units. Rederivation techniques will also be applied to protect and improve the health status of model organisms across the academic institute.

4. The archiving and if necessary, the distribution of roughly 180 mouse lines per year over the duration of the project.

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 production of genetically altered animals to act as model organisms enables studies into the function and interaction of genes over a broad range of subject areas [REDACTED] Mouse lines will be maintained in a suitable clean environment that is

protected from undesirable disease. Appropriate management strategies for each individual model will be implemented to ensure efficient mouse production. The use of sperm or embryo cryopreservation and assisted reproductive techniques, provided through an experienced and optimised core, will enable and expedite the implementation of the highest standard line management practices. Furthermore, cryopreservation as part of good management allows for the efficient shut down and if required, the rapid expansion, of a line whilst resetting the genetic integrity to the point at which the line has been archived. It also enables efficient distribution to collaborators and sustainable repositories, preventing the unnecessary breeding and transportation of live animals. Recent advances in the field of assisted reproductive techniques permits optimised routes for the introduction of models into specified pathogen free barrier animal units, resulting in a reduction in the overall number of animals required during the importation process that is essential for protecting the health status of existing animals within the academic research establishment.

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

This license require the use of mice as a mammalian model. The project will last for a duration of 5 years and is expected to require the use of up to 40000 mice.

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?

Due to the nature of this project, the anticipated adverse effects within this license are likely to be very mild i.e. through Breeding and maintenance of GA mice and Superovulation protocols. The creation of novel genetically altered lines can exhibit adverse phenotypes however genetic targets will be selected to reduce severity. Surgical protocols must be considered moderate. Comparatively mice within the moderate band would total less than 15% of the total animal usage. Control measures will be implemented wherever possible to reduce adverse effects e.g. by using aseptic technique during surgery. All mice on this project will either move onto another project license for continued use, or will be culled by a schedule 1 method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Creating genetically altered animals for use as models permits the study of the functions and interaction of genes across broad subject areas, such as Cancer or Diabetes studies. Advances in gene manipulation have resulted in the creation of animal models which contain mutations which can be tissue or time specific.

The recent arrival of CRISPR/Cas9 technology provides a currently unparalleled precision and speed when creating novel genetically altered lines.

Mice are a good species for scientific research due to sharing a high percentage of genetic material with humans. As a mammalian model it mimics human physiology, organ structure and tissues as well as replicating genetic milestones and disease conditions. Due to the complexity of interactions within whole organisms the use of animals is in some cases unavoidable.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The centralised production, breeding and archiving of genetically altered mouse lines provides an opportunity to analyse working practices in order to use the fewest animals to achieve the intended aims.

By exploiting transgenic technology including the recent advances utilising programmable nucleases such as CRISPR/Cas9, it will be possible to generate novel mutants much more efficiently. Increases in targeting efficiency will result in fewer founder animals and reduced time frames.

Archiving mouse lines will inherently reduce the numbers of animals required for any given project and when completed pre-emptively, will allow for efficient line removal and if necessary expansion.

The archiving of mutant lines will predominantly be accomplished through the cryopreservation of sperm, which is significantly more cost effective with regard to animal usage than cryopreserving embryos. Breeding and maintenance of mutant lines for distribution to various research projects will permit centralised colony management, reducing the breeding and managed excess required whilst ensuring the best husbandry and genetic integrity.

Distribution of novel transgenic lines to the scientific community will potentially allow for a global offset against production rates by reducing the necessity to recreate lines elsewhere.

Refinement

Explain the choice of animals 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.

Refinement

The mouse, as a mammalian model, mimics human physiology, tissue and organ structure, as well as many genetic milestones and disease states. Knowledge that can be attained by studying genetically altered animals helps to study disease activity with or without the use of therapeutic agents.

By centralising core components of mouse production, archiving and assisted reproductive techniques we can most efficiently implement the best working practices across all projects and subject areas. Continual review of gene targeting and integration success will ensure that models are being created efficiently and the thorough validation of cryopreserved archives will also ensure there is no unnecessary animal cost when recovering as a live resource. Breeding performance will be regularly reviewed and the best management techniques implemented to optimise performance.

PROJECT 94

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Evaluation and genetic control of immune mechanisms in ruminants
Key Words	Immune mechanisms, REDACT?, genetic diversity, Mastitis
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aim of this project is study how sheep respond to infection and immunisation and to understand why some animals respond better than others. We plan to develop tools that will allow us to analyse the immune cells that initiate and control immune responses against pathogens and the genetic basis of immune variation in sheep populations. These studies will support the development of vaccines that protect against infectious diseases in livestock

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 disease is a major constraint to livestock health and welfare and adversely affects the efficiency of the production system contributing to significant waste. The control of disease through vaccination will have substantial benefits in through increased efficiency of the production system, improved health and animal welfare as well as over all reductions in green house gas emissions.

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

Over the five years of this project we expect to use between 1000 and 1400 animals. The vast majority of these animals will be sheep which will be blood sampled on a single occasion to provide a DNA sample for population genotyping.

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?

Obtaining a blood sampling from REDACT? is a mild procedure with no adverse affects. The majority of these animals will continue to live on the commercial farms.

A small number of animals with specific genotypes will be selected for immunization, vaccination and challenge studies. The majority of these studies are mild in nature requiring blood sampling to investigate immune cell populations. We also plan intra-mammary immunization and challenge experiments which may be moderate in severity in some animals. Depending on the study animals will either be maintained on [REDACTED] or if tissue samples are required animals will be humanely destroyed using a regulated procedure.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The purpose of this licence is to improve our understanding of genetic diversity and the induction of immunity in livestock species in support of the development of new and improved vaccines. Current genotyping protocols require a high quality sample of DNA and or mRNA which is most simply obtained from a single blood sample. Non-invasive alternatives do not currently provide nucleic acid of the required quantity and quality. The development of tools to study the induction and characterisation of cellular immune responses in livestock requires cellular material which currently can only be collected prior to and following infection or immunisation.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

All animal studies are planned following consultation with independent statisticians in order to minimising the number of animals in each experiment.

Refinement

Explain the choice of animals 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.

Refinement

REDACT? are an important component of the UK agricultural sector and infectious diseases of sheep constitute a significant constraint to animal health, welfare and productivity. We are focused on the development of disease control methods for REDACT? livestock species which requires a range of sampling, inoculation and

infection procedures. We have developed and continue to refine the immunisation and infection models in-order to ensure consistent mild to moderate disease with only transient discomfort to the animal. All animals are frequently monitored and maintained in state of the art animal care and holding facilities and are under the care of trained veterinary surgeons and animal technicians.

PROJECT 95

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Physiological biomarkers of REDACTED welfare.
Key Words	Neuroscience, acute affective state, electrophysiology, REDACTED
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Increasing welfare of REDACT? (and other farm animals) is high on the public agenda. However, understanding which conditions or management processes affect the animals more negatively is difficult to ascertain. We should not assume that, just because humans might (not) like certain conditions, that REDACT? would respond accordingly.

We therefore have to ask the REDACT?. This can sometimes be done with behavioural tests, but there are a number of situations in which it is impossible to use behaviour, because the animal is unable to behave normally (e.g. when being picked up and put into crates for transport).

This project aims to use physiological (neurobiological) indicators as potential measures of the animals' welfare state. We aim to develop short-term, immediate measures, which give us an idea of an animal's immediate emotional state. For this, we are looking at brain activity in brain areas that are known to process emotions.

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)?

If we can validate these markers, we can use them to assess the welfare impact of different management practices on the animals. If we can assess this, then we can make practical recommendations as to which methods are higher welfare than which others. Because we are doing this research in collaboration with a company that designs processing systems for REDACT?, our findings will be implemented quickly.

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

We will use REDACT? of 4-7 weeks old. Over the 5-year length of the project, we anticipate using 100 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?

Because we need to validate that our measures can identify negative experiences, we need to induce these experiences in the animals. However, these are unlikely to be any worse than those they might encounter had they been kept in commercial establishments. For a few animals, some aspects of their environment may be worse than they would normally have experience. However, the potential benefit to the millions of REDACT? being housed and then killed every year in the UK alone will outweigh the slightly increased negative experiences of a small number of REDACT? will undergo surgery under general anaesthetic to implant electrodes into the brain. They will be allowed to recover from anaesthesia and heal from surgery before being recorded. Post-surgical pain will be treated with routine analgesics. The recording will be conducted by attaching a wireless recording device to the implant. This will be designed to be as light as possible and to impair the animals' movements as little as possible, so that the impact on the animal is minimal, and we can focus on the impact of the different stimuli we present to it. These stimuli may include negative stimuli, such as brief restraint, brief periods of pain or bad-tasting food; or the can be positive stimuli, such as re-uniting them with flock mates, providing dust baths or providing them with preferred food types. If necessary, the animal will be given time to habituate to the equipment before we start recording. At the end of each experiment, the animals will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Because we are interested in the physiological responses of live animals to different environments and conditions, we have to study this in live animals. No in-vitro or computer model can mimic the response of real animals. However, early optimization of the implant surgery will be done first with cadavers, and then with non-recovery anaesthesia before moving to recovery surgeries.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use the most powerful statistics available to make sure we can use the fewest possible animals for the most possible outcome. Whenever possible, we will conduct power analyses to estimate the minimum effective sample size needed. The experiments will be done within- REDACT?, allowing us to control for a lot of inter-individual variability, and therefore to reduce the sample size needed to obtain meaningful results.

Refinement

Explain the choice of animals 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.

Refinement

Because our question is about REDACT?, we have to answer it using REDACT?.

Except for the experimentally required negative experiences, we will minimize the animals' negative experiences by closely monitoring them for distress and disease, and by administering antibiotics and/or analgesics when necessary to the animals recovering from surgery. We will use wireless recording methods for the electrophysiology, as this reduces the stress on the animal of being physically connected with a wire.

PROJECT 96

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Production of high antibody plasma
Key Words	High antibody horse plasma
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To produce high antibody equine plasma of sufficient high quality to be safe and efficacious to transfuse into foals.

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)?

Primarily, neonatal foals can be transfused with equine plasma to prevent septic disease and therefore prevent the pain and suffering that would cause. Secondly a proportion of the herd of donor horses would otherwise be put to sleep as they would not have any other purpose they could fulfil.

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

Approximately 65 horses over 5 years

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

This activity is no different to human plasma donation and is considered mild in terms of severity and therefore very rarely is any significant or long lasting adverse ef-fect expected. A small number of instances of local reac-tion to vaccines or blood sampling is possible all of which are mild and have very little adverse impact on the welfare of the REDACT?. The horses will be kept at the end of the project either for re-use in the same project again or re-homed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Due to the structure of the REDACT? placenta antibodies do not cross into the REDACT? during pregnancy and it therefore relies on passive transfer of immunity in the first few hours after birth from its first ingestion of colostrum. This passive transfer of immunity does not always occur successfully and such REDACT? are much more susceptible to septic disease in the first few weeks of life. It is well documented in the veterinary literature that REDACT? plasma from suitable donors administered intravenously can remedy this situation.

In addition, in certain situations, specific septic disease becomes established on REDACT? farms and in the absence of vaccines or alternatives it has been recognised that equine plasma containing specific antibodies to the causal infection administered to REDACT? at or soon after birth contributes to the significant decrease in the incidence and severity of such disease.

It is not possible to produce this in other species for transfusion into foals due to incompatibility, nor is there any synthetic alternative. In summary it improves the welfare of REDACT? and reduces the economic loss.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

This project work consists of establishing and maintaining a herd of suitable donor REDACT? in manageable groups free to graze but with adequate accessible housing with supplementary food all the year round. The REDACT? are vaccinated, blood sampled and their plasma harvested in compliance with strictly controlled parameters to ensure welfare and safety of the animal. Their health and welfare is assured by daily health checks and the employment of best practice routine veterinary preventive medicine procedures. They are inspected by the Named Veterinary Surgeon in compliance with current regulatory requirements and their continued welfare assured by additional veterinary attention as required. Incremental gains are achieved in maximising donor horse health to make the most of production at each donation, monitoring systems are utilised to avoid wasted procedures. Together these steps reduce the number of procedures required and also the number of REDACT? overall.

Refinement

Explain the choice of animals 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.

Refinement

The procedures used to harvest plasma closely mimics plasma collection from humans and thus in terms of severity it is accepted as being so mild that suffering is not considered to occur. During plasma harvesting food is always available and in all cases the donor REDACT? feeds at will during the plasma harvesting process.

It is anticipated that up to 65 REDACT? will be used for this project. Records from previous projects indicate there are no adverse effects from harvesting plasma under the established protocols with the animals being managed under natural conditions for the whole of their natural lives and kept in an exemplary culture of care.

Detailed records of each pheresing procedure are maintained along with detailed individual REDACT? health records which are used to manage each animal prior to and during each pheresis.

PROJECT 97

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Understanding and targeting the tumour microenvironment
Key Words	Cancer, immune system, inflammation, immunotherapy
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Cancers are not just masses of malignant cells but a rogue organ of a variety of cells that are recruited and corrupted by the malignant cells to help the cancer grow and spread. This complex mixture of cells is known as the tumour microenvironment. Understanding this tumour microenvironment is critically important to developing effective approaches for cancers that are currently difficult to treat. We are especially interested in finding combinations of treatments that more effectively disrupt interactions between malignant cells and the other cells that support their growth, especially treatments that stimulate the immune system to fight the cancer.

The project will focus mainly on ovarian, pancreatic, kidney and lung as well as cancers of B lymphocytes. These are all diseases where new treatments are urgently needed.

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 potential benefits of this project are new and more effective treatments for human cancers that currently cause suffering and loss of life. In addition, our experiments will increase our knowledge of the tumour microenvironment and of how malignant cells recruit and corrupt normal cells to help the cancer grow and spread.

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

The estimated number of animals to be used over the 5 year duration of the project in all the protocols is 38,000. Animals to be used are mice. They are the least sentient species that allow the objectives to be met. Up to 50% of the animals used will be harmful mutants (immune-deficient animals or genetically-altered animals).

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

We will manipulate the mice so that they develop cancers either by genetic manipulation or injection of cancer cells into relevant sites in the body. These cancers will have a strong resemblance to the human disease that they model. Expected adverse events: bodyweight loss, lung metastases, liver metastases, splenomegaly, spontaneous tumour development, ascites formation, adverse reaction to some treatments. Expected level of severity: Moderate. Measures taken to limit harms: frequent monitoring of disease-specific clinical signs and non-specific clinical signs for early identification of adverse events, moderate signs tolerated for no more than 24 hours, severe signs not tolerated. Humane endpoints are applied to minimise harm and include humane culling prior to the development of severe clinical signs. At the end of an experiment, all animals will be culled.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The tumour microenvironment is a dynamic mix of cells that evolves over time. Cells are recruited to the cancer from the surrounding area, from the blood and bone marrow. Although we are now developing complex human tissue culture models to recapitulate this, we cannot replicate the dynamic interactions that happen in a living animal. However, we are now making extensive efforts to develop tumour microenvironment models that grow in the lab. For instance we are building a model of human ovarian cancer using patient material obtained at surgery as well as a model of cancer blood vessel development. Within the next five years it is our intention that these models will replace some of our experimental mouse models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We ensure that we obtain a maximum amount of information from each mouse that we use using imaging during the experiment and then taking extensive samples at the end of the experiment.

We conduct pilot experiments to optimise the experimental design. Where necessary we conduct power analysis or consult our in-house biostatistician to make sure that

we have enough, but not too many, mice in each experiment to give us significant results.

We will always consider archiving of genetically modified lines as frozen sperm or embryos to reduce the number of living mice we have to maintain and to allow sharing of these lines between researchers, providing further opportunities for reduction.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the least sentient species that allow the objectives to be met. We use mouse cancer models that replicate the human disease as closely as possible. We use our knowledge of the human cancers that we study to make sure that the mouse cancer models are relevant.

We house the mice in individually ventilated cages to avoid any infections.

We have extensive experience of working with genetically altered mice so we are alert to health problems and can take timely action to minimise suffering, to ensure pain relief is quickly administered whenever necessary and we have clear guidelines on humane endpoints.

PROJECT 98

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Chemosensory mechanisms underlying the homeostatic control of internal states of the body
Key Words	breathing, body weight, obesity, apnea
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We wish to understand how the brain senses the body's internal state with respect to: 1) carbon dioxide (CO_2) and the regulation of CO_2-dependent processes such as breathing and blood flow in the brain; and 2) circulating nutrients and the control of appetite, fat deposition and bodyweight.

For CO_2-sensing we wish to understand the role of a protein called Cx26, which has previously been shown to be a direct sensor of CO_2. We shall explore the role of this molecule in detecting CO_2 in the brain and how detection of CO_2 via Cx26 interacts with detection of the acidity of blood to give coordinated control breathing and blood flow in the brain. We shall also examine the effect on the control of breathing and brain blood flow of human pathological mutations that affect CO_2-sensitivity of Cx26, paying particular attention to whether the effects of the mutations on the control of breathing could depend on whether the person is awake or asleep.

For the control of appetite, fat deposition and bodyweight, we shall examine how key glial cells called tanycytes in an area of the brain called the hypothalamus are activated following a meal, and whether this follows the time course over which the concentration of key nutrients (glucose, amino acids) change in the brain. We shall determine how tanycytes detect key nutrients and pass that information onto hypothalamic neurons known to control bodyweight. We shall examine if tanycytes are sensitive to changes in food intake such as short term fasting, restricted amino acid intake or a high fat diet. We shall test the hypothesis that tanycytes communicate a "stop signal" to the feeding networks that could depend on how well fed that individual is.

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

This project will provide significant advances in fundamental knowledge and mechanistic understanding in the following areas of neuroscience. 1) How sensing of acidity and CO2 are integrated to give complete control of breathing and brain blood flow. This may be particularly important if one of the mechanisms of this reflex is already saturated e.g. during a state of chronic blood acidity arising from respiratory or cardiac pathologies. 2) How detection of key nutrients such as amino acids by hypothalamic cells contributes to the control of food intake, energy expenditure and body weight. The regulated excretion of CO2 via breathing is a critically important life preserving process. Understanding how this is achieved and how it may vary during sleep and wakefulness will have great benefits for human health. For example, mutations of Cx26 cause deafness in 1:2000 people. As some of these mutations affect its ability to detect CO2, they may lead to weaker breathing. By understanding more about how Cx26 contributes to the control of breathing, arousal and brain blood flow we should be able to predict which mutations could cause unexpected additional health problems, such as central sleep apnoeas, in deaf people. Sleep apnoea is linked to the UK's biggest killers e.g., cancer, heart disease/failure, stroke, and liver disease. 10-25% of the population suffer from sleep-disordered breathing and identifying patients that may suffer from sleep apnoea early could lead to timely preventative treatment that would improve their quality of life and have a large impact on the NHS. Obesity rates in the UK are approaching 33% for adults and 10% for children. Forecasts suggest this could rise to >50% for adults and 25% of children by 2050 if nothing is done. The current direct burden to the NHS is estimated as £4.2b per year. Obesity greatly increases the risk of diabetes: within the UK, nearly 2.7 million people were type II diabetics in 2010. Diabetes UK estimates 10% of the NHS budget (£9bn) is spent on diabetes and its complications (e.g. hypertension, heart disease, Alzheimer's disease and others). Our work is likely to suggest mechanistically informed approaches to help people avoid becoming overweight or to lose weight.

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

We shall use rodents (rats, mice) and we expect to use about 1000 animals over 5 years.

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

Many procedures will be non-invasive –such as behavioural testing or monitoring of breathing. We may need to perform surgical procedures and we shall constantly monitor the animals to make sure that they do not suffer, and that any distress is kept to a minimum and within the limits defined in the project. There are obviously possible complications of general surgery such as infection, pain or haemorrhage.

We shall reduce these complications by making sure everyone on the project is extensively trained in how to perform the procedure and animal care. We also have pain medication and antibiotics readily available to treat animals when it is needed. We also work very closely with a veterinarian and other highly trained technicians to make sure that the animals are well looked after and do not suffer in any way. At the end of the experiment all animals will be humanely culled.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

This project seeks to understand: the control of breathing; arousal; cerebral blood flow; and the contribution of sensory mechanisms in the hypothalamus to the control of feeding behaviour and body weight. As these are phenomena that arise in the whole organism they can only be studied in whole animals and tissues taken from animals. It would be unethical to use human subjects in these experiments.

Whenever possible we shall use reduced systems *in vitro*, or *ex vivo* tissue to test the cellular and molecular mechanisms involved in signalling. This will enable replacement of animal usage and will permit us to use animals to perform only the most essential experiments to develop fundamental physiological understanding.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We shall use a staged approach in our investigations.

Firstly, where possible we shall use *in vitro* experiments to formulate specific testable hypotheses as to their roles for investigation *in vivo*. This will allow focussed and optimal experimental design.

Secondly, we shall utilize pilot experiments to validate and optimize stimulation protocols and experimental design. This will also enable us to calculate the numbers of animals required to achieve definitive conclusions in out experiments.

Thirdly the logical structure and order of our experimental questions is designed to prevent needless procedures if the result of an investigation proves negative.

We shall invest in new equipment to further improve the accuracy of our virus injections thus reducing in the number of viral injections in which the target nucleus was missed or off-target nuclei hit. This will reduce animal usage.

The nature of the genetic modifications will allow us to perform repeated measurements on the same animal, thus reducing animal usage. This will be especially true for the behavioural experiments as we can perform multiple behavioural tests on the same set of animals, thus reducing the total number of animals used.

Wherever possible we shall perform terminal anaesthetised experiments on animals used in chronic studies thereby reducing animal usage. These experiments will also allow us to gather data to guide our chronic awake behaving experiments, and thus will improve our overall experimental design.

Refinement

Explain the choice of animals 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.

Refinement

Rodents are a well-established model for studying the neural control mechanisms of breathing, cerebral blood flow, appetite and energy homeostasis. This gives a robust literature and wealth of potential data obviating the need to repeat past findings. Rodents in general are seen as a simplified and experimentally tractable model for far more complex mammals such as humans.

Wherever possible, physiological testing of phenotypes will be achieved by using non-invasive methods such as: whole body plethysmography for detection of breathing movements; and fMRI for blood flow; classical appetitive conditioning for food preferences. More invasive procedures will be used only when there is good reason (from the non-invasive experiments) to expect mechanistic insight.

Advice and training on viral transduction will be obtained from collaborators and researchers highly experienced in these methodologies and we shall follow the Laboratory Animal Science Association guidelines on performing aseptic surgery to ensure best practice for the recovery surgery involved in all surgical procedures.

PROJECT 99

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	TESTING AND DEVELOPING AQUACULTURE HEALTH PRODUCTS
Key Words	bacteria, virus, vaccination
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The objectives of this programme are to improve the health and welfare of cultured fin fish, whilst providing a service to test the safety, efficiency and potency of existing and developing health products. The first part of the programme is to develop, refine and validate viral and bacterial challenge models of infection to aid in the development of treatments of cultured fin fish species. The second part is to provide a service offering test systems and challenge models to pharmaceutical, feed and aquaculture companies to provide data on safety, toxicity, pharmacological behaviour, efficacy or biological activity of veterinary products and feed additives in compliance with European guidelines; thereby aiding their development for use in the Aquaculture Industry.

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

1. Fish in farming systems will have improved welfare due to a reduction in disease outbreaks through the use of safe and efficacious products 2. Feed, vaccine and pharmaceutical companies will increase their range of health products for the aquaculture industry 3. Fish farmers will have a wider selection of products to improve their fish health management strategies 4. The impact of antibiotics and chemicals on the environment will be reduced through the use of efficacious vaccines and immunotherapies. 5. The consumer by having access to healthier fish produced through more sustainable aquaculture practices.

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

[REDACTED]

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?

1. Adaptation, refinement and validation of fish infection models Severe 2. Passaging pathogens moderate 3. Dose response determination of pathogen challenge dose Severe There is a high likely incidence that a challenge in with bacterial/ viral pathogens Protocols 1, 2 and 3 will result in mortality/morbidity. 4. Efficacy challenge test Severe Bacterial/viral challenge of treated/untreated fish is necessary to determine the relative efficacy of the test product. Typically, up to 80% of the control fish and a lower proportion of the treated fish may be affected and will develop a systemic infection, which will result in mortality or morbidity. There is a high likelihood that a challenge with bacterial/ viral pathogens will result in mortality/morbidity. Safety Test Moderate Administration of substances by voluntary feeding, immersion, gavage, injection or spray may cause adverse effects which could include morbidity, abnormal appearance and behaviour (including feeding behaviour) and histological changes in up to 100% of the treated fish. Fish exhibiting signs described above will be removed from the study by a schedule 1 method. Residue depletion test Mild It is unlikely that adverse effects will be noted as the fish would be exposed to the compound at the safe dose for the calculated field rate in its target species. Toxicity Test Severe The likely incidence of adverse effects is high. Adverse effects may include mortality and morbidity, abnormal appearance and behaviour (including feeding behaviour), histological changes. Lethal and sub-lethal effects may be expected in up to 50-70 % of fish treated with the test material. Animals in all protocols will be euthanized using an appropriate schedule 1 method at the end of each experiment.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

It is necessary to use live fish to confirm the efficacy of vaccines and vaccine candidates and confirm the efficacy of dietary additives/immunomodulators, probiotics, chemotheraputants *in vivo.* They are the target species for these products and there is currently no other model available for this work.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Studies will include the use of replicate tanks with fewer fish to increase the statistical power of any studies, whilst reducing fish numbers. Where possible, fish would be randomly allocated to their treatment tanks and a blocked, random design for the study produced. Being mindful of the need to assess fish welfare and behaviour, we would anticipate where possible, the use of blinding in our studies. Provision will be made to account for potential mortalities and withdrawals during a trial to avoid the need to repeat experiments which will be planned with statistical robustness in mind. For *in vivo* work we often use the tank as the experimental unit and include three replicate tanks for each treatment in order to ensure that useful data are obtained without repetition due to tank effects

Refinement

Explain the choice of animals 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.

Refinement

Fish are the necessary model for this work for this work as they are the target species for the products to be tested and there is no other model currently available that can be used for this work. Fish must be challenged to determine the efficacy of the treatment relative to an untreated control group. The work will be conducted in accordance with the European Pharmacopeia where appropriate. Although some procedures involved are classified as severe fish will be sacrificed as soon as suffering is detected

PROJECT 100

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	CILIOGENESIS AND CELL POLARITY IN ZEBRAFISH
Key Words	Cilia, Polarity, Ciliopathies, Degeneration, Zebrafish
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Our aim is to determine how cilia-related genes function in various aspects of cell biology, including proliferation, survival, metabolism and the detection of extracelluar signals. Cilia are protrusions present on the surface of cells in many species, including man. Defects of ciliary function lead to a number of human disorders including disruptions of organ laterality (situs inversus), formation of additional digits (polydactyly), kidney cysts (polycystic kidney disease), blindness (retinitis pigmentose), mental retardation, infertility, and others.

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

We will study the structure and function of cilia in genetically altered embryos of a freshwater fish, Danio rerio, commonly known as the zebrafish. The results of our analysis will reveal genes and proteins that can be potentially targeted by therapeutic compounds or used in gene therapy in order to eliminate or minimize disease symptoms of human cilia-related disorders.

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

Over the period of five years we will use up to 45100 zebrafish to study these phenotypes.

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

The experimental protocols that we will use are designed to minimize suffering. When necessary, we use anesthesia to eliminate pain. As the zebrafish is the simplest vertebrate genetic model organism, its pain perception is poorly developed, compared to other vertebrates potentially available for this analysis. To further reduce discomfort potentially involved in experimental procedures, our studies will be performed on embryonic and larval forms of zebrafish, which have relatively poorly developed nervous system. At the end of experimental procdures the animals will be humanely euthanized.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We have considered the use of invertebrates, however invertebrate and vertebrate ciliated tissues are very different and therefore we have chosen to use zebrafish such that our findings can be translated to higher vertebrate organisms including man.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have extensive experience from previous work in designing experiments in a way that reduces the number of animals involved. We have access to a dedicated statistician who helps with our experimental design to ensure that we use the minimum number of animals required per experiment to conclusively answer the questions posed.

Refinement

Explain the choice of animals 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.

Refinement

The zebrafish is the least advanced of all genetically tractable vertebrates and we can also take advantage of its transparency during development to perform detailed image analysis to gain greater insights in to cilia function. Animal suffering will be minimised by using embryos and early larvae wherever possible. Aneasthesia will be used whenever possible.

PROJECT 101

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	VIRAL VACCINES (RESEARCH)
Key Words	Vaccine, Virus, Disease
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The objective is to make the materials and perform tests to be able to assess the quality and effectiveness of new and existing biological products, such as vaccines. These tests are essential to ensure the vaccines are safe and effective before being administered to 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)?

One part of the work will be to develop tests that can replace the use of animals and in one case a test in rats that could replace a test currently performed in primates. Other tests could lead to improvements in vaccines so that are better at preventing disease and help to make vaccines for new diseases or for existing diseases that do not currently have effective vaccines. Safety tests of viruses used in biological products could improve their safety or potentially reduce the time taken to make urgently needed vaccines The consequences of using a vaccine of low potency or inappropriate strain are that it will fail to protect recipients and disease burden in the human population could increase.

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

Mouse 4500 Rat 3000 Rabbit 100 Ferret 550 Chicken 20 5 years Mice are used because they make good immune responses to many test materials and there are a large range of commercially available materials to analyse the responses. Rats, REDACT? and for some tests ferrets are used because it is a regulatory requirement to use a particular animal for that test. Rabbits are used if a large quantity of serum is needed to make material for an in vitro test. Ferrets are used for influenza tests because the immune response developed and the illness they experience both closely resemble those seen in humans.

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

Animals may be injected with substances by the intraperitoneal, intramuscular, subcutaneous, intradermal or intravenous routes, or dosed with substances by the intranasal route. Newly born rats will be injected intracranially with mumps viruses. Some animals will have a microchip implanted under the skin for the purpose of identification and to monitor temperature. Samples may be collected e.g. blood, nasal washings, or mouth or eye swabs. Injection and dosing procedures, microchip implantation, and sample collection procedures are expected to cause no more than mild and transient discomfort. Where appropriate anaesthesia is provided to limit distress. Repeated anaesthesia may be given to immobilise animals for non-invasive procedures e.g. imaging. For animals being immunised there may be some local irritation at the site of inoculations particularly where adjuvants are used. Any animal showing signs of adverse effects as a result of the regulated procedures will be humanely killed unless there is a rapid return to normal using no more than minor medical treatment. Some animals will be infected with influenza viruses and will experience influenza like illness. If possible animals that become ill will be treated with medicines to alleviate symptoms according to a regime recommended by the vet. Ferrets and mice infected with virulent influenza may become seriously ill, experiencing weight loss and impaired movement and may be at risk of death from the disease unless there is prompt intervention. Where possible animals will be treated with anti-viral medicines to prevent development of serious illness. The outcome of these infections can be unpredictable and so animals will be monitored very closely by experienced staff with knowledge of humane end points. Any animal that has any significant adverse effect will be humanely killed using an overdose of anaesthetic. All animals used under this licence will be humanely killed at the end of the study, or before if it is necessary for the welfare of the animal.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The data concern the immune response and in some cases the protective immune response to viruses and vaccines and the pathogenesis of disease, which cannot be generated without the use of protected animals.

In some cases data will be generated to validate *in vitro* assays with a view to eliminating the use of protected species.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The types of experiment proposed have been conducted for many years and the numbers of animals required in each approach to give a successful outcome are well established by experience. Where appropriate, statistical input is sought on animal experiments so that the numbers of animals used are the minimum needed to produce statistically reliable results. Sometimes the numbers used are based on regulatory requirements, for example to test a vaccines strength or for safety tests

Refinement

Explain the choice of animals 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.

Refinement

Ferrets are among the few animals other than primates whose response to infection with influenza reflects that of humans; both the immune response and the clinical signs closely resemble that seen in humans. Methods for observation of clinical signs have been developed for recognition of onset of disease allowing earlier intervention with the use of anti-viral drugs or medication to relieve symptoms or to identify and humanely kill animals before progression to severe disease states .

It is recognised that group housing is preferable for optimum well-being of ferrets and wherever possible they will be group housed. There are situations where single housing is required due to husbandry needs or for safety reasons. In these situations wherever possible animals will be housed in cages in rooms with other ferrets.

Best husbandry practices will be employed to reduce the possibility of rejection of the rat pups by their mothers. Mothers and pups will be closely observed following injections and any rejected pup will be humanely killed immediately

Anaesthetics will be used for procedures where there is potential to cause pain or distress to an animal

Immunisation with adjuvants suitable for use in humans will be used. Freund’s adjuvants will no longer be included for use under this licence. Studies under previous versions of this licence have established that alternative adjuvants are at least as effective in achieving the required outcomes.

PROJECT 102

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Amphibian egg extracts as tools for biomedical research
Key Words	DNA replication; genetic stability; cancer
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Genetic instability is a characteristic of a large proportion of cancers. This can take many forms, but frequently involves loss or duplication of lengths of DNA, or movement of these sequences from one chromosome to another. These problems are most likely to occur when the DNA in a cell is being copied (replicated), before the cell divides. The aim of the research programme is to understand the pathways used by normal cells to prevent over- or under-replication of chromosome segments. We will investigate mechanisms to avoid or repair these mutations, or failing that, to induce a cell containing them to die without further division.

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 basic experimental part of the programme will use Xenopus frog egg extracts, which provide a strong base for understanding the basic biochemistry of genome stability pathways, and human tissue culture cell lines, where the role and importance of different pathways can be explored. We will apply this basic research to understanding the development of oesophageal cancer, which shows very high levels of wholescale loss or duplication of DNA sequences. This work may lead to the development of biomarkers to identify premalignant changes that are at high risk of becoming cancerous (and should therefore be treated promptly), or markers to aid choice of the best treatment regime when malignancy is detected.

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

600 Xenopus frogs over 5 years

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

The frogs experience only transient discomfort when injected to induce egg-laying. Frogs remain in the egg-laying colony for 4-5 years, after which they are killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Cell-free extracts of *Xenopus* eggs provide the only currently-available eukaryotic system capable of supporting efficient cell cycle progression in vitro. They allow extremely tight control over cell cycle status, and allow chromatin to be isolated with no biochemical disruption to the system. Therefore there is no reasonable alternative for the experiments described in this proposal. The lack of alternative model systems and the minimal suffering involved in egg production justifies the continued use of animals for this work. Although the cell cycle can be studied in vivo (such as in C. elegans, yeast, Drosophila or in tissue culture cells), it is not possible to perform biochemistry in living cells.

added by Applicant 6 minutes ago

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

By maintaining the colony in excellent health and in good condition, we maximise the yield of eggs and minimise the number of animals required. After eggs are laid we prepare cell-free extracts which we freeze down for future use, thereby maximising the use we can make of the material.

Refinement

Explain the choice of animals 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.

Refinement

Our procedures have been refined to ensure only minimal and transitory discomfort to the animals on injection.

We are currently exploring the use of an alternative procedure for inducing egg-laying in Xenopus, where progesterones and oestrogens are added to the water in the tanks where the frogs are kept for egg-laying. We have found that this can yield significant quantities of eggs, though the results appear to depend heavily on environmental conditions such as temperature and lighting. If successful, this protocol could completely replace subcutaneous hormone injection, meaning that *Xenopus* egg laying (used in many biochemical and developmental research projects) would be significantly less stressful to the frogs.

As well as trialling hormones in the water, we may also trial ways to improve egg quality or laying and/or frog welfare at these times by manipulating environmental conditions (such as by manipulation of temperature, light quality or timings, water flow, tank colour/size/shape, etc. These manipulations (other than around tank colour/size/shape will be within the conditions tolerated by this species in the wild and changes will not be sudden or extreme. We hope that this will allow us to maximise the quantity and quality of eggs laid so that the minimum numbers of animals are used in the least stressful way

PROJECT 103

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Novel therapies for disorders of the immune system
Key Words	immune, disorders, inflammation, novel, therapeutics
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The objectives of this project are to validate drug targets for treating immune and inflammatory diseases, to identify new drugs for treating immune and inflammatory diseases, and to test for the side-effects of new drugs that may occur in the inflammation response system.

Compounds in early drug discovery research will be tested in rat and mouse in vivo models inflammation models.

The process of inflammation is part of the body's immune response to the presence of pathogens, irritants or damaged cells. The inflammation response removes the harmful stimuli and initiates healing. While the inflammatory process is essential for healing wounds and infections, there are many immune and inflammatory diseases, which usually occur when the immune system mistakenly initiates inflammation in the absence of infection, such as inflammation of the joints in rheumatoid arthritis.

Abnormalities associated with immune and inflammatory processes underlie a wide range of human disease states, a lot of them with obvious associated inflammation, such as allergies or myopathies. However, many additional diseases are now also known to have their underlying cause in disorders of immune and/or inflammatory processes, such atherosclerosis, ischaemic heart disease, diabetes and cancer.

Current treatments for immune and inflammatory diseases come from a variety of drug types, each of which are associated with significant side effects, such as severe gastric effects and an increased risk of opportunistic infection due to prolonged immunosuppression.

This project covers protocols designed to assess the effect of potential novel drugs on immune and inflammatory responses in vivo, with an emphasis on rheumatoid arthritis and dermatitis.

Experiments in this project typically follow test tube (in vitro) studies. However, in vitro methods cannot predict and replace whole animal (in vivo) models, as the technology does not exist to simulate the complexity of the whole body system. New, potential medicines that do merit testing are assessed in rat and mouse 'models' of human immune and inflammatory diseases that detect clinically effective medicines.

The in vivo rat and mice experimental protocols used by this project model immune and/or inflammatory responses and associated disease symptoms. The symptoms are generated by exposure to specific agents that produce immune responses like those observed in human pathology. Immune responses are measured by analysing changes in the level of immune mediators response released in vivo, tissue inflammation and resulting physical symptoms. Animals are typically tested in protocols that last 1-5 days, but occasionally animals may be tested for up to 70 days.

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

The major potential benefit of this work is the discovery of novel drugs for the treatment of immune an inflammation diseases, areas of unmet medical need. This project licence will enable drug discovery projects that are aiming to produce treatments which are superior, in terms of effectiveness and side-effect liability, to current treatments.

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

Rats and mice will be used on this licence, as they are the lowest sentient species that can be used. As an estimate, a novel compound will be tested at 3 doses with 2 controls per experiment (a vehicle treated group and a clinically relevant control) with 8 animals per group at a rate of three experiments per month. This would equate to a total of up to 8400 rodents over the 5 years' of the project.

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 those associated with testing novel compound from early stage drug discovery projects (eg mild sedation and salivation), but the likelihood of occurrence is low. Close monitoring and use of pilot studies, will help to keep the incidence of adverse effects to a minimum. Possible adverse effects associated with inflammation disease models include joint and/or swelling and stiffness, with consequent reductions in movement, and wheezing. Animals will be monitored closely to ensure they can have free access to food and water and do not lose weight. All animals will be humanely killed at the end of protocols.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The in vivo models described in this licence application are employed to generate information about how the whole body responds once it has been given a compound. It is neither possible, nor ethical, to use human volunteers in early drug discovery. It is therefore necessary to use other whole body systems, animals, to find out how a living organism responds.

The studies covered by this licence typically follow on from in vitro models/assays performed by our clients. The in vitro models provide useful information about which are the best chemical leads from a particular chemistry program to be select for further study.

However, at present, in vitro methods cannot entirely predict and replace the in vivo models described by this licence, as the technology does not exist to simulate the complexity and diversity of the whole body system.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals required per group and experimental design are determined on the basis of power analysis, advice from statisticians, published data and previous results that have consistently identified target effects in a clear and unambiguous manner.

- Whenever possible repeated measure analyses will be employed to increase precision, maintain smaller group sizes, and reduce animal usage.For example behavioural measures from one subject might be recorded, and compared, both before and after drug administration, or on multiple occasions over time.
- Within each experiment a positive control is included to provide an internal control to compare the relative efficacy of the test compound and to assess the sensitivity/validity of the test procedure on a given test occasion. This good experimental design principle will avoid unnecessary replication of experiments.

Refinement

Explain the choice of animals 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.

Refinement

Purpose bred, adult free living animals of assured health and genetic status will be obtained from commercial suppliers, or from breeding colonies.

Animal suffering will be minimised by the following;

- Conditions in the animal house follow current best practice, and items such as bone chews are placed in rodent cages for their stimulation.
- Competent personnel will perform all studies on this project licence and adverse effects will be minimised by careful handling and the application of good technique.
- Guidelines on the limit of volumes of administration of substances and blood sampling will be strictly adhered to.

Clear-cut end points are described in the possible adverse event description for the protocol covered by this licence.

PROJECT 104

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Cellular response to stress and injury in the intestine
Key Words	intestine, inflammation, Colorectal cancer, Microbiota, infection
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Inflammation and cancer are the main disease processes that affect the intestine. In both cases there is an interaction between the cells lining the bowel wall, the gut nervous system, the immune system and the microbes in inside bowel. The objective of the proposed experiments is to unravel the complex interactions between the cells lining the bowel , immune system, gut nervous system and gut bacteria during infection, inflammation and cancer. These interactions are crucial in development of Inflammatory Bowel Disease, Necrotising Enterocolitis, colorectal cancer and gastrointestinal infections.

The aims of the project is to determine i) how the intestinal invasion of microbes and poisonous substances is regulated by the immune system, gut microbes, the metabolism of the bowel, ii) how the growth and loss of the cell lining the bowel is regulated iii) how the gut microbes colonise the bowel in early life and influence inflammation and cancer and iv) how the intestinal barrier is regulated by the nervous system of the gut.

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 form the essential ground work for the development of new diagnostic tests and therapies for inflammatory bowel disease, necrotizing enteritis, infective gastroenteritis, the irritable bowel syndrome and colorectal cancer. The discoveries from this project will be used to develop new tests for example the use of measures of gut permeability to determine the severity of inflammatory bowel disease or to enable the interpretation of the significance changes in the gut bacteria analyzed in clinical practice. Through this programme we hope to identify healthy gut bacteria

that are therapeutic in for inflammatory bowel disease, necrotizing enteritis, infective gastroenteritis and colorectal cancer. The scientists involved in this programme work with the pharmaceutical industry and use these results for the development of new bacterial-based therapies.

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

Over the 5 year period of this licence a maximum of 11150 mice will be used. Around 50% of these will not undergo any scientific procedures, but will be used solely for breeding and maintenance of colonies.

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

We expect no more than moderate discomfort from our surgical procedures, tumour implantations, or substance administrations. At the end of each procedure, all animals will be killed by a schedule 1 method. When we wish to administer substances, we shall use either gene therapy procedure called hydrodynamic tail vein injection or a very small pump the size of a small pill inserted under the skin under anaesthesia. At the end of each procedure, all animals will be killed by a schedule 1 method. For experiments investigating how bacteria from the intestine of the pregnant mother affects to the new-born pup we shall study bacteria in the milk of the mother and use caesarean section and rearing by a foster mother to tease out the origin of bacteria in new born pups. The experiments carried out on pregnant or lactating female mice are not expected to cause any harm to these mice or to their unborn pups and neonates.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Prior to embarking on animal experiments we will collect as much evidence as possible to determine whether a candidate gene or microorganism is likely to regulate intestinal epithelial cell shedding / barrier function or immune response. These in vitro experiments inform our *in vivo* experiments allowing us to limit the number of animals used. However *in vitro* assays cannot adequately model the complete array of signalling pathways involved in the interplay of microbes, epithelial cells and immune system which are important in gastrointestinal disease. Therefore, further *in vivo* work is required.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

When designing experiments we perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative. As a general principle, for quantitative experiments, sample sizes will be set using power analysis. Generally, the significance level will be 5% and the power 90% after advice from a statistician.

In order to reduce the number of breeding pairs, the mice will be kept as homozygous mice wherever possible, provided they do not have a harmful phenotype.

To maximize the information from a single animal, we will aim to collect samples from multiple body sites and provide those samples to appropriate scientific colleagues.

Refinement

Explain the choice of animals 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.

Refinement

We use mice because they are genetically altered in the expression of proteins we believe are critical for intestinal barrier function and response to bacteria. Where possible C re-loxP systems are used to specifically target the tissue of interest which reduces off-target adverse effects in the animal. Our protocols are routinely examined to ensure they are up-to-date and incorporate current best practice. Where surgery is required we employ analgesia to ensure minimal discomfort. We use early humane end-points for all studies. Some of our experiments impact on the health of the mice for example, induction of colitis and the spread of cancer from the bowel to the liver. In all cases detailed statistical advice is taken to minimise the number of mice studied consistent with scientifically valid results. In the case of colitis mice are sacrificed any severe symptoms or signs develop. In the case of cancer spreading to the liver, mice do not experience much ill effects from the liver cancer but can develop ill effects from the bowel cancer. In this experiment the mice do not develop wide spread cancer. Once again the mice are sacrificed before any severe ill health develops.

PROJECT 105

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Physiology of mammalian hearing system: Interaction between sensory and supporting cells and extracellular structures
Key Words	Cochlea, Hearing physiology, Hearing loss, Hereditary deafness
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production

	conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To develop successful treatments for hearing loss, including restoration through regeneration, replacement and development of specialised prostheses, it is necessary to understand the complex electromechanical functional relationships between cellular elements of the mammalian organ of Corti, and between these elements and the major extracellular matrices, in the healthy and impaired cochlea. The major objective of this research proposal is to understand this interaction especially with respect to apparent differences in sensory processing between the apical low-frequency and basal high-frequency regions of the cochlea.

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 proposed research is centred on using novel techniques for addressing several topics that are foci of intense interest in the field of auditory biophysics and therapy. Outcomes from this research will provide data for feedback models of the mechanics of the cochlea, including our own. This is an iterative, inclusive, process that is aimed at providing new, exciting, and productive directions in the field of auditory research, centred on the cochlea. Significantly, it will provide a deep understanding of the workings of the cochlea that is a necessary foundation to providing effective measures for the repair and replacement of sensory receptors in profoundly hearing impaired cochleae. Accordingly, outcome from the proposed research will be of significant importance to research efforts, including those in our own labs, directed at discovering ways to restore profound hearing loss and in the early detection of hearing loss.

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

2500 (500/yr) mice and 250 (50/yr) guinea pigs over five 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?

Animals produced under this project are not expected to exhibit any harmful phenotype. The level of severity for most of the procedures used in this project is either unclassified or mild. The level of severity can approach moderate in experiments with induction of conditional gene expression. At the end of the research procedures animals will be killed by a Schedule 1 method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animals have to be used for these experiments because the study of function of normal cochlea and cochlea which suffers from genetic disorders is the purpose of the project. In vitro hearing organs are not yet available. Any attempts to isolate the cochlea for in vitro study would compromise its normal function and would result in severe increase in thresholds of its neural and mechanical responses.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Our experiments are technically challenging, but we have a high success rate of > 80%. The essential feature we are looking for is repeatability of measurements. We can usually achieve this objective in measurements from 10 -20 preparations to give us repeatable measurements from 5 – 10 preparations that fall within the 95% confidence limit of a mean. Preparations that fail this test do so because of surgical errors made during preparation (very rare) and physiological failure, which resulted in a limited data set. Because the mammalian cochlea is not readily accessible and because experimental manipulations to the cochlea are technically challenging, our experimental data are used to develop and refine computer models of the cochlea. Simulation of cochlear responses using these models will allow for reduction of animals used in future experiments.

Refinement

Explain the choice of animals 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.

Refinement

The guinea pig cochlea has been used extensively as a model of the human cochlea and is relatively accessible to complex, physiological, investigation. Mice are the most appropriate animals to use to meet these objectives as they are the only species for which germline-transmitting embryonic stem (ES) cells are available. We have developed special techniques to make mechanical measurements from the cochlea of mice.

All procedures will be carried out using anaesthesia. In all cases, except those employing non-invasive, non-traumatic assessment of auditory function (measurement of distortion product otoacoustic emissions), the animal will be killed by anaesthetic overdose or via perfusion fixation at the end of the procedure.

PROJECT 106

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Investigations and Research into Steroid Hormone Metabolism using Mouse Models
Key Words	Hormones; steroids; metabolism;
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Steroid hormones exert a diverse array of actions, a number of which impact on how energy is used and stored in the body. As such, disturbances in steroid levels can contribute to metabolic diseases such as diabetes, obesity and fat infiltration into the liver. Currently we know little about how steroid levels within tissues such as liver, fat and muscle are regulated. There is considerable interest in regulating steroid availability for the future development of drugs to help treat these conditions

The impact of steroids on health is not only due to their direct effect on tissues, such as liver and fat, but also on how different tissues interact. We have begun to build a picture of the local effects of steroids but need to expand our understanding of how they impact these tissue-tissue interactions.

Therefore, our two key objectives are; to identify novel ways by which steroid hormone action is regulated within tissues and to determine how the responses of specific tissues to steroids impacts on other tissues to drive conditions such as diabetes and obesity.

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

Our studies will help to understand the pathways within cells by which steroid hormones act and the impact that this has on the interactions between different tissues. It is hoped that this will allow us to identify novel drug targets for the prevention or treatment of metabolic disease.

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

We will use mouse models, as mice provide rapid and efficient breeding and have a high enough degree of similarity to man to be translatable to human disease. We expect to use approximately 6300 mice over the five years duration of the licence.

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

The maximum severity experienced by mice on this licence is moderate, however, the majority of mice are expected to experience of only mild severity. Likely effects due to alterations in steroid action or dietary challenge are obesity, diabetes, and liver disease. The adverse effects experienced by any mouse will be monitored closely. All of the mice will be humanely killed at the end of the procedure.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Development and progression of metabolic diseases requires the interaction of multiple tissues, in particular liver, fat, muscle and gut. In addition, the regulation of steroid hormone and bile acid levels requires the interaction of the hypothalamus, pituitary and multiple target tissues. As this represents a whole body system, it is not possible to investigate and understand disease development in isolated cells and therefore bearing in mind these complex interactions, it is important to study whole live animals. In addition, the effects of drugs need to be tested in whole animals so that the responses of all the different organs, and their interactions, can be studied. However, we are able to study some of the direct effects of steroids and bile acids in cell models. In particular we are able to measure how fats and sugars are used and stored within cells and how this is controlled. This work will inform our animal studies and may be able to replace mice in some preliminary studies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

In all our mouse work, we use statistical analysis to ensure that the minimum number of mice are bred for the study, and that we use only the number of mice that are required to produce meaningful and useful results in order to answer the experimental questions. We are able to study the effects of genetic modification on multiple organs within an individual animal, for example in mice which develop

metabolic disease in more than one organ. Similarly, we can carry out different protocols on the same mouse across the study, rather than using several mice once.

Refinement

Explain the choice of animals 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.

Refinement

Only mice will be used in our studies, and have been chosen as they represent the lowest mammalian species that allow the necessary genetic manipulations and display sufficient similarity to human organs and physiology. We have developed expertise and experience in mouse welfare, and have refined our tests to ensure that the highest quality data is generated for the least welfare cost.

We are keen to minimise severity and increase the welfare of these animals. To ensure this, we will use non-invasive tests that only cause temporary discomfort where possible. For administration of drugs, a small pilot study will be undertaken for new drugs, with increased cage observations and welfare checks to ensure that the drug is safe. We also aim to use long acting drugs where possible to reduce the frequency of dosing. During every test, mice are closely observed and anaesthetics or analgesics used when appropriate.

PROJECT 107

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	The evaluation of veterinary medicinal products
Key Words	Evaluation, Efficacy Studies, Vaccine, Medicinal
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To provide data for the licencing process for veterinary medicinal products, this primarily focuses on proving their safety, quality and efficacy. [REDACTED]

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

The work carried out under this licence aids the development and licensing of new medicinal products and improves currently licensed products, thereby contributing to improving animal and human health, food production and the control of infectious diseases. There is also an economic benefit to the consumer and farmers through more efficient production. These products are fundamental in controlling disease and the spread and effects of infectious pathogens in animals and humans.

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

This licence is demand led, the first protocol is for work on a Bovine Viral Diarrhoea 2 (BVD2) virus vaccine which will involve 24 calves for 3 months. Second experiment is assessing safety of two poultry vaccines and requires 60 hens and last approximately 16 weeks.

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?

There should be no adverse effect of the vaccine, for the unprotected control group BVD 2 should have a transitory illness with signs such as inappetence, elevated temperature, moderate depression, mild nasal discharge and loose faeces. The REDACT? experiment is mild, there should be no adverse effects of the vaccine but the trial is to prove safety so health and laying performance will be monitored. The

REDACT? will be euthanized at various predetermined time points to meet regulatory requirements.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The response to inocula such as pathogenic organisms and vaccines is a very complex process and one that cannot be replicated *in vitro.* It is not possible to generate definitive safety and efficacy data without the use of animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used is often specified in the regulatory guidelines so there is limited scope for reducing the number of animals. However, scientific expertise, input from customers, discussion with regulators and statistical rigour ensures the number of animals used is minimised. Possible reduction is discussed with the Sponsor and adjusted according to the aims of the studies.

Refinement

Explain the choice of animals 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.

Refinement

Although study designs are often prescribed by regulators, careful consideration is given to possible refinements on a case-by-case basis. For example in batch testing it may be possible to not challenge vaccinated animals, relying on the measurement of antibody response which eliminates the development of disease. In addition, data from companies and their communications with the regulatory authorities and feedback directly from the competent authorities may allow tests to be refined. All plans undergo review by the Institute's Animal Welfare and Ethical Review Body. All require close monitoring of all animals used in Studies. Detailed Clinical Monitoring Schemes with clear humane end-points and actions are prepared for all those involved. These allow treatment or euthanasia at the earliest possible time and ensure they do not exceed the severity limit of the protocol.

PROJECT 108

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Control of Bacterial Products Used in Medicine
Key Words	Vaccine, Biological medicine, Safety, Quality
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Immunological medicinal products such as vaccines are tested by an independent laboratory before being released onto the market. These tests, some involving animals, help to ensure the quality of these products.

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 principal benefit from this project is the assurance obtained that batches of immunological medicinal product are likely to be safe and effective for use in humans. A high level of assurance is particularly important for vaccines. If public confidence in vaccine safety/effectiveness falls, vaccine uptake and coverage may be reduced and diseases that were previously well controlled by immunisation can re-emerge. In many cases, the target population is healthy infants and children and the tolerance for adverse effects from prophylactic medicinal products such as vaccines is much lower than for other medicinal products. Consequently, the regulation of these biological medicines is rigorous and necessitates the use of animals for some of the tests that are performed by the independent regulatory laboratory.

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

The project involves use of small animals only (mice and guinea pigs). For the duration of the project (5 years) it is expected that up to 13,704 animals will be used (930 guinea pigs and 12,774 mice)

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

The project includes 12 protocols, of which 2 are mild procedures, 8 are moderate procedures and 2 are severe procedures. The majority of animals are not expected to experience more than moderate adverse effects (for example irritation or inflammation at the site of injection, or signs of general toxicity such as loss of appetite, weight loss and reduced activity). Frequent monitoring and supportive husbandry measures are used to minimise the impact of these adverse effects. For the 2 protocols with a severe severity limit, both involving mice, adverse effects may include shock, convulsion, weight loss, trauma (as a result of brain injection), and loss of consciousness. In some cases death occurs for some of the test animals (up to 20% across these two protocols). At the end of all tests animals are humanely killed using a schedule 1 method. The impact of these adverse effects is mitigated as far as possible by rigorous observation of the animals by experienced staff and the application of recognised humane end points where necessary.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The tests used in this project are performed according to methods described in regulatory documents such as European Pharmacopoeia monographs and WHO recommendations or are based on methods included in product licence dossiers. In all cases, no suitable validated non-animal alternatives are currently available. Where validated alternative test methods exist, they have been introduced and this has resulted in a removal of some protocols from this project licence. Efforts are continuing to develop and validate alternatives to some of the methods that are retained in this project licence.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

For the tests used in this project, the number of dose groups and size of each group is based on well-established methods that are described in regulatory monographs and guidelines. Some procedures have been modified to reduce the number of dose groups and/or number of animals in each group based on experience gained with the procedure and the advice of an experienced biostatistician. Many tests require the inclusion of a reference group and one or more control groups and where possible, test samples will be included together in a single assay to maximise the use of these reference/control groups and therefore minimise the total animal use during the project.

Refinement

Explain the choice of animals 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.

Refinement

The tests included in this project involve the most refined end point possible – for example, serological assays as opposed to direct challenge assays. Frequent monitoring by experienced staff, including out-of-hours checks for some procedures help to ensure that welfare costs are minimised wherever possible. For the two tests with a severe severity limit additional refinement measures include the use of anaesthetic during a challenge procedure for one test and the use of dermal temperature monitoring as a second end point which helps to avoid the need for repeat testing where results obtained from the primary end point are inconclusive. As part of routine and ongoing animal welfare measures, animals will be housed in groups in caging suitable for the species used, with a range of varied and appropriate enrichment to allow natural behaviours.

PROJECT 109

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Evaluating anti-metastasis and anti-tumour strategies
Key Words	Cancer, therapies, spread, progression, bone
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The spread of cancer to the bone represents an incurable phase of human cancer disease development. This is a poorly understood phenomenon with little or no curative current therapies. Additionally, the spread of the disease to the bone and other sites around the body frequently results in painful lesions with a loss of quality of life and often associates with resistance to currently used clinical therapies.

The aim of this project is generate animal models of human cancer, which recapitulates this end stage of human disease. This will enable us to evaluate and test novel therapies targeted against cancer disease spread with special regards to the bone. It will also highlight potential genes involved in these processes which will expand our understanding, provide novel therapeutic targets and guide future research.

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 models in this project will be used to test experimental cancer treatments affecting many stages of the human disease. The aim of our research is to shorten the timescale for developing new drug to treat human cancers. Our specific models and protocols will ensure that the novel drugs will be tested in a highly relevant manner, thus allowing the reduction of animal numbers compared with existing techniques. To discover novel targets involved in the process of spreading to other sites around the body, for future drug development. We also hope to develop the next generation in ultrasound guided surgical tools to treat solid tumours (particularly breast tumours). This is of particular importance to the elderly with breast cancer as general anaesthetic is not always advisable, or those who cannot undergo surgery. This technique could be used under local anaesthetic leading to reduced risk to the patient and lower cost in hospital stays.

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

Mouse 4000 (800 per annum) over 5 years

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

Adverse effects will be the development of cancers both as primary tumours and disseminated disease. Tumour studies will be moderate in severity, i.e. the animals are likely to experience short term moderate pain, suffering or distress,which will likely cause moderate impairment of the wellbeing or general condition of the animals. Breeding of genetically modified animals will be mild in severity, i.e. animals are likely to experience short term mild pain, suffering or distress. Procedures with no significant impairment to the wellbeing or general condition of the animals. The mice will be killed at the end of the study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Development of new anticancer drugs requires evaluation of efficacy in animal tumour models prior to clinical trials. The interactions with bone and blood vessel cells cannot be truly recapitulated *in vitro* due to its inherent complexity. Therefore, *in vivo* work is needed to determine the therapeutic potential of lead molecules. The rodent xenograft models are widely regarded as the most appropriate and least severe to evaluate new anticancer drugs. Nevertheless, *in vivo* assessment is only carried out following rigorous testing of potential targets and/or drugs using *in vitro* assays and in cell culture model systems.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Basic screening of compounds and targets will be done *in vitro* prior to any animal work.

The experiments are designed to produce maximum statistically valid results from the minimum of animals.

Basic laboratory experiments can be used to test targets/compounds for mechanisms of action and efficacy, as well as to evaluate haow they work and how well they could be used in treatment. This will limit the number of animals

as we will only use compounds/targets that are most appropriate and well-defined. This helps to ensure that the fewest possible number of compounds/targets are taken forward to animals studies and hence reduces the number of animals used. Moreover, longitudinal imaging (Protocol 1) will lead to further reduction and the animals will not be killed at each time point.

Refinement

Explain the choice of animals 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.

Refinement

Nude mice with compromised immune systems allow the growth of human tumours for drug evaluation – this ensures that human-relevant targets are used. The nude mouse is valuable to research because it can receive many different types of tissue and tumour grafts, as it mounts no rejection response to any human cells introduced into its system.

For some transgenic animals only mouse strains are currently available.

Mice will be housed in individual ventilated cages to minimise infections. Adverse side effects and general health will be monitored throughout.

Humane endpoints will be used for all experiments.

Anaesthesia and analgesia will be used wherever appropriate.

PROJECT 110

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Crosstalk between inflammation, cell death and cancer
Key Words	Inflammation, immunity, cancer
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Cell death and inflammation are important processes which are constantly happening in our body. Yet, they need to be well regulated and require a tight control so that their beneficial effect is not turning into a maleficent happening. Upon infections or mechanical damage such as injury of skin or organs our immune system reacts by causing an inflammation and immune response against that damage and the tissue repair system will eventually restore normal conditions after the healing process is accomplished. Cell death which can occur either unwanted as a negative side effect in an organ/skin injury scenario or as a wanted process to kill e.g. viral infected cells, plays an important role in this process. Too much cell death or an overshooting immune response in our body can result in a reaction against itself and thus can cause a variety of diseases such as autoimmunity, autoinflammation and cancer. Still, the mechanisms leading to these deregulated events mentioned above are poorly understood. In order to develop new drugs for these diseases, it is essential to understand their origin i.e. what is causing the deregulation of events and to characterise the key components involved in this, their activities and how they interplay. To this end we will study how inflammatory and immune pathways correlate with cell death and genes controlling the formation of cancer. In addition, we intend to evaluate the effect of potential inhibitors for specific proteins or activities within a protein that are key for the regulation of such pathways.

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 order to prevent or reverse life threatening diseases such as chronic inflammation, autoimmunity and cancer new therapeutic treatments are needed. The use of

genetic mouse models is crucial to understand these pathways and to test potential inhibitors that could increase human and animal welfare and even be life-saving.

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

In order to achieve this we will need approximately 40000 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 levels of severity? What will happen to the animals at the end?

Some of the animal models we intend to use spontaneously develop an inflammation of the skin also known as dermatitis. If we are not able to revert this condition by genetic experiments or chemical/biological treatments the animals will be humanely killed as soon as they start to show signs of distress (hunchback position, excessive scratching, etc). Other animals develop swollen lymph nodes; this condition is not harmful for the animal and they will be either bred or used in experiments. Animals will be humanely killed as soon as enlarged nodules restrict movement or any other physiological condition. Some other adverse effects may arise by the application of chemical or biological substances into the mice. The animals will be closely monitored during these procedures and will be humanely killed as soon as they start to show signs of distress (hunchback position, lack of grooming, excessive loss weight, etc).

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

All of the planned work with mice (referred to as in vivo work) is generally preceded by intensive studies done in the laboratory with cell lines only (so called in vitro work). Before carrying out any experiments in the mouse we will make sure to monitor for as many physiological conditions as possible in our various cell lines. Yet, although the in vitro work can provide important molecular and cell physiological insight it unfortunately cannot fully recapitulate the complexity of a pathophysiological situation in the context of inflammation and cancer in a mouse or a human.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Thorough in vitro experiments and in depths studies (see above) will allow to limit the number of animals required for the in vivo investigation as most key components involved in inflammation and oncogene-driven cancer can be identified in cell lines.

Importantly, a strict management of mouse colonies will minimise the number of animals bred. For this reason one person will be exclusively occupied full time on taking care of this important task.

Further reductions in the number of mice will be achieved by improved study design and statistical methods which will allow us to integrate and aggregate the data across multiple experiments thus achieving a statistical conclusion which makes additional experiments with animals superfluous.

Refinement

Explain the choice of animals 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.

Refinement

Choice of species

The overall plan of this work is to first, understand what leads to deregulated cell death, inflammation and inappropriate immune responses which can result in autoinflammation, autoimmune diseases and/or cancer and second, to develop novel therapies to benefit human health in targeting these diseases.

For all investigational work in this project mice are the species of choice. This is due to the fact that the murine immune system and the different modes of cell death happening in mice are well-studied and many features are comparable to the human system. Furthermore, numerous well-defined models for all of the diseases we are aiming to investigate in this project are available. Using genetically-altered (transgenic) mouse strains is currently the most effective way to investigate a specific gene function at the level of the whole animal. The application of commercially available products (specific inhibitors for different proteins, small molecules etc.) in our transgenic mice will allow us to investigate the murine immune system and its reaction/response at the most refined level currently possible and to translate our results into the human system.

Minimisation of suffering

All of our procedures are designed to minimise pain to the animal as much as possible. Yet, certain procedures will have a moderate impact on the wellbeing of the animal. Thus, we have developed pre-defined endpoints to pre-empt and avoid the

onset of any adverse effects and to keep the negative impact on their welfare as low as possible. These pre-defined endpoints take different parameters into account such as behavioural changes, body weight etc.

In the procedures where we induce tumours for instance the pre-defined endpoint is reached as soon as the tumour burden appears to impair its mobility and the mice show signs of distress such as lack of grooming and separation from the group.

The parameter which define the endpoint for the procedures which induce ulcerative colitis include signs of distress such as excessive weight loss and hunchback position.

In every procedure we try to keep the length of time for each experiment to a minimum.

Additionally, a close monitoring of animals throughout any kind of treatment or of those which are genetically prone to develop a phenotype will guarantee that they are not suffering excessive adverse effects.

Concerning the mice which are prone to develop diseases due to their genotype (e.g. the mice which develop inflammation of the skin), we have established a breeding strategy which allows us in many cases to delay or completely prevent the onset of disease by combining certain gene mutations thus contributing to the welfare of the animal.

In addition, the institute in which the animal procedures are carried out, runs a comprehensive health-monitoring programme. Animal health and welfare records are maintained to include any adverse effects that may develop, particularly in genetically altered and spontaneous mutant strains. Signs consistently associated with a particular phenotype/genotype will be recorded on the respective “information sheet” in the breeding area and the mice will be maintained under conditions where their health status can be protected as far as it is reasonably practicable.

PROJECT 111

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Immunogenicity of Crimean-Congo Haemorrhagic Fever virus vaccines
Key Words	Crimean Congo Haemorrhagic Fever Sheep
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

There is an unmet need for safe and effective vaccines for livestock against Crimean Congo Haemorrhagic Fever (CCHF). CCHF is a disease spread by ticks from livestock to humans, and has a fatality rate of 15-70%. It is present in many countries, including Asia, Eastern Europe and southern Africa. It is, at present, an increasing problem in Western Europe including areas such as Greece and Turkey. Assessment of a vaccine which can prevent and control CCHF in sheep is the aim of this study.

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 information gathered from this study will allow the vaccine to be used in REDACT? in countries where CCHF is present. Preventing and controlling CCHF in livestock in these countries will help to control CCHF infection of humans, and therefore ultimately help prevent the loss of human life associated with this disease.

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

In this study we expect to use around 100 REDACT? in 5 years.

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

Following vaccination of the sheep with the CCHF vaccine, no adverse effects are likely / expected other than shot lived discomfort / elevated temperature as is normal with any vaccination, and is considered to be of a mild level of severity. All animals will be euthanized at the end of the study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

REDACT? are the target species for this vaccine, in that they will be the species in which this vaccine will be used out on farms and in the field in countries where CCHF is present. Due to the complex nature of the immune system, it is not currently possible to assess with certainty how good an immune response a vaccine will illicit from pure in vitro analyses. Furthermore, before this vaccine is permitted to be used on farms and in the field in endemic countries, information on how effective it is in REDACT? is required by the regulatory authorities of the country in question.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use statistical tools to determine the minimum sample size that allows determination of our study endpoints with appropriate power.

Refinement

Explain the choice of animals 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.

Refinement

This vaccine has already been shown to be effective in mice, however due to REDACT? being the species in which this vaccine will be used in to control CCHF in endemic countries, it is most appropriate to use sheep as the animal model for this programme of work. Animals will be housed together with bedding and other items of enrichment. Highly trained animal technicians will monitor these animals throughout the day, ensuring they are comfortable and to maximise their welfare status.

PROJECT 112

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Study of muscle response to physiological stimuli.
Key Words	Muscle, stem cell, sarcopenia, physiology, regeneration
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Skeletal muscle is a large human tissue that responds to various environmental and physiological stimuli by changing its size and function.

For a large number of diseases like muscle wasting conditions, metabolic and degenerative disorders, skeletal muscle size and function are compromised.

The aim of our work is to identify genes that regulate muscle physiology and can ultimately be targeted for therapeutic uses to potentially treat muscle wasting and degenerative diseases in humans. For this purpose we will examine the physiological response on genetically modified animals to environmental challenges including exercise, diet and tissue injury.

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

Our studies have the potential to advance biological knowledge and improve our understanding about the molecular pathways that affect muscle homeostasis and function in the context of several degenerative disorders due to ageing or metabolic challenges. Our studies have the potential to identify disease mechanisms that may offer novel therapeutic targets.

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

7000 wild-type and genetically modified mice will be used for the implementation of the proposed work. This is based on our ongoing experience of these experiments.

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

We will breed genetically modified mice that will be subject to exercise protocols or nutritional changes or tissue damage. The severity for one protocol involving tissue damage is moderate. Pain relief and strictly aseptic surgical conditions will be followed. Animals will be humanely euthanised at the end of experimental procedures in order to proceed with the harvesting and analysis of biological tissue.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animal tissue is a complex organ that responds to environmental influences in a number of ways and as such whole organ studies are not suited to mathematical or computer simulations and cannot replace the use of experimental animals. Animal testing for efficacy and proof of principle is needed before any form of clinical trial on human subjects. Therefore, animal testing is necessary for ultimate assessment of the efficacy and safety of regimes that alter system physiology. The culturing of skeletal muscle in an in-vitro setting has been established for many years. However the major drawback of these types of experiments is that they do not present a good model for mature mammalian myofibres due to size, shape and metabolic limitations. Therefore presently we do not have appropriate in-vitro models that mimic body muscle fibres.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The proposed experiments have been carefully designed using statistical power calculations according to the relevant scientific literature. This approach allows for reliable sample size estimation in order to keep the animal numbers to a minimum without compromising the scientific quality of the proposed work. The use of minimum animal numbers will be assured by:

1. Often our preliminary data allow us to predict the outcome of new experimental series.

2. We will be checking for statistical trends continuously and as soon as significant differences are evident for the required power of 80% we will not use additional animals.

3. Multiple tissues of similar scientific interest from the same animal will be subjected to a battery of different analytical techniques further reducing the requirements for more animals.

Refinement

Explain the choice of animals 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.

Refinement

The mouse is the lowest sentient mammal which can be used in this type of study to inform on the human condition. This is due to the fact that mouse muscle uses the same molecular and cellular programmes during muscle development and regeneration. Other experimental non-mammalian models, e.g. fruit flies, nematode worms and zebrafish, do not share these characteristics with mouse. Their physiological response to exercise, diet and injury is very different to that of mammals. Furthermore the mouse is the only organism in which the differing genetic manipulations have been made to target the genes of interest. In all manipulations we will carry out a sequence of experiments that is designed to minimise the suffering of advent of adverse conditions. Examples include:

1. Genotyping of mice will be done using the less painful ear punching instead of tail clipping.

2. For the exercise protocols we will use the least severe form of exercise first and only move to the next form of more forced exercise if the animals do not voluntarily run.

3. To avoid hypothermia during general anaesthesia a heating pad will be used.

4. Any clinical signs that might emerge such as accidental injury to the paw during treadmill running, or infection/inflammation to injection sites will be immediately reported to the veterinarian in charge for further action. Possibly transferred to another regime once healed.

5. Littermates that do not express the transgene will be used as control wild type animals and animal colonies will be reviewed regularly to avoid the production of surplus mice.

6. Aseptic surgical conditions, use of pre-surgery anaesthesia as well as post-surgery analgesics and all the necessary actions to attenuate animal suffering and

discomfort are going to be scheduled in collaboration with the veterinarian of the School.

PROJECT 113

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Inflammation, immunity and tissue function
Key Words	Inflammation, Infection, Tissue damage, Normal function
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The consequences of inflammatory diseases are considerable in both developing and developed countries alike. Inflammation is important for the control of infection and wound repair, but can negatively impact on the normal function of our tissues, particularly when dysregulated or prolonged (chronic). The objectives of this project are to better understand the processes that drive appropriate inflammatory responses and also that maintain normal tissue function, the overlap between them and how one process when dysregulated or modulated may negatively or positively impact on the other.

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)?

A holistic view, should identify novel therapeutic approaches to the regulation of inflammation and restoration of tissue function. Hence as a primary benefits we anticipate the further development of potential targets regulate inflammation and tissue repair.

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

Mouse, 9,000 (largely through complex breeding programmes) over the course of the project.

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

The majority of the experiments will be mild in severity. They will involve one of a few stimuli that evoke a self-resolving transient inflammatory response, which will be

studied with various interventions to understand the processes occurring. Some procedures will involve stimuli of moderate severity, where clinical signs of adverse effects will be more evident or longer lasting. In some cases we will study mouse models of spontaneous chronic disease development, to understand how they develop and how we may intervene. In all cases, the presence of adverse effects is specifically monitored for and in most cases mice will be killed if exhibiting evidence of such effects.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Wherever possible we have developed *in vitro* systems for the addressing our specific questions, for example producing our own cell lines as a direct replacement. Cell lines do not display all the characteristics of the cells found a living animal and whilst cell culture techniques have dramatically improved, the distribution of multiple cell types and the presence of many diverse cell:cell interactions cannot be replicated *in vitro*.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

As part of our standard procedures, every experiment involves, prior to commencement, an assessment of the design. This including statistical analysis or equivalent where possible of the number to be used. This ensures the correct numbers of animals are used to be able to have a realistic chance to address the scientific question.

Refinement

Explain the choice of animals 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.

Refinement

A mammalian species must be used because of the complexity of biological systems studied. Mice are also the most appropriate species because of the advantages of genetic manipulation in mice. We refine our procedures by titrating doses, administering substances by the least adverse methods and using low-dose

challenges first. We will add to this by, for example, exploring the use of implanted minipumps as an alternative to repeated injections. We also use animal welfare scoring systems with ‘humane experimental end-points’ to limit any suffering and risk of adverse effects.

PROJECT 114

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Musculoskeletal biomechanics of vertebrate motion
Key Words	locomotion, evolution, bird, reptile
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We seek to better understand how animals move using their musculoskeletal system, in diverse species from reptiles to birds, and in diverse behaviours including walking, running, jumping, turning and standing up. We aim to use a combination of experimental measurements and computer simulations to do this.

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 study will help us to improve the simulation approaches used (testing their limitations or assumptions and accuracies), and to reconstruct how the diversity of locomotor behaviours evolved in land vertebrates. This will advance basic scientific knowledge and open new potentials for applications of simulation-based methods (improved by our analyses) or clinical/robotics innovations (inspired by our findings of how unusual animals work).

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

REDACTED 10 of each; sub/near-adults, over a period of 5 years of research. None of these animals are endangered and all will be sourced from UK/EU breeders.

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

Animals will be trained do the behaviours of interest in an experimental laboratory setting. They will then undergo moderately invasive surgery to implant tiny (<1mm; small relative to the animals) measurement devices (variable in number from approximately 6 to 15) into their muscles or bones, allowing us to measure at the highest level of precision possible how their locomotor systems function during these behaviours. Experimental data will then be analysed in computer simulations to

calculate how un-measurable factors such as muscle forces and contraction speeds generate the observed behaviours. The animals may show some low levels of lameness which may reflect modest levels of pain. However, a goal of the work is to minimize pain, which is undesirable because it would lead to altered behaviours and thus more difficult interpretations. The best analgesia will be used to ensure that pain is minimized. Overall the severity levels will be moderate. Animals will be monitored to give them time to recover from surgery, then will be used for about 1 week of experimental analysis each and then euthanized. Their cadavers will be used for the simulation part of the project, and 3D anatomical data from the underlying models, as well as experimental data, will be shared openly so that future research may benefit from it. We have the expertise to care for these animals and provide appropriate accommodation and social conditions.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Live animals need to be studied because little is known about the species of interest (most research is on “model” species such as mice, humans, horses, cats, chickens and guinea fowl) We cannot advance knowledge sufficiently using cadavers alone, as we need to measure real behaviours in order to determine how animals move. Cadavers do not have operative nervous systems and active muscular responses and thus cannot locomote, which renders them useful as preparatory subjects for this study but not useful for testing the study’s main hypotheses. Furthermore, as anatomical form and biomechanical function or behaviour are not always closely linked, predicting the latter from the former is not considered reliable in modern science; hence cadaver-based studies have severe limitations. Even in model species, behaviours other than walking and running are often little studied, and direct measurements of what the muscles do to generate those behaviours are yet fewer. A major goal of this study is to refine computer simulation approaches so that they are more accurate and trustworthy and thereby can replace experimental measurements more often, reducing the use of animals, but those approaches are still in dire need of direct experimental tests before this could happen.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Sample sizes are kept low (~10 animals/species) and the statistical power of the methods is generally high. Furthermore, large numbers of animals are not needed

because the questions being asked do not demand the ability to detect tiny differences- the behaviours of birds, for example, are easily distinguished at the detailed levels of investigation that this study involves.

Refinement

Explain the choice of animals 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.

Refinement

Exemplars of reptilian and avian species have been chosen based on availability, suitability (considering prior experience with them). Furthermore, the species span much of the diversity of locomotor form, function and behaviour in land vertebrates from more REDACTED to bipedal and athletic birds. Both species chosen retain many features considered to be primitive (evolutionarily ancestral) within its group and thus they are good choices for evolutionary or comparative studies, not being overly specialized relative to other species in their lineage and thus poorly representative. To minimise welfare costs to the animals, we will provide care and monitoring by our expert veterinary clinicians at all times and ample food, water and rest throughout captivity. Analgesia will be provided as required.

PROJECT 115

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Development and validation of patient-derived xenografts to advance treatments for prostate cancer
Key Words	Prostate cancer, cancer stem cell therapies, personalised medicine, near-patient cancer models
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aims of this project are to better predict which patients with prostate cancer will benefit from certain treatments and to identify the cells that are resistant to cancer therapies.

The treatment of advanced prostate cancer remains disappointing despite recent clinical success. We are now beginning to understand that cancer is a very heterogeneous disease, and that we have to tailor treatments more effectively. Reproducing this complexity in a surrogate, animal model is the focus of this project with the long-term aim of improving patient outcome.

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

There is now growing evidence that ‘patient derived xenografts’ (propagating a patient’s tumour in a mouse) mimic the response to treatment(s) observed in patients. The potential clinical and scientific benefits of establishing a model that encompasses the heterogeneity of prostate cancer include: identifying groups of patients who will benefit from a particular treatment and understanding why patients become resistant. This mouse model is also likely to be of benefit to the pharmaceutical industry. New drugs often fail, despite early promise, because the current models do not reflect the complexity of this disease.

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

Mice, 1500 over 5 years

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

The surgical procedures experienced by the mice in this project will be no more than moderate severity. The mice will develop prostate cancer and the tumours may grow to a size to cause minor discomfort, in which case they will be given pain-relieving medication. The level of severity will be no more than moderate. Animals will be humanly killed at the end of the experiment and their tissues banked for further analysis. For procedures in which the effects of anticancer agents are being evaluated the maximum tolerated dose shall not be exceeded such that the level of severity shall be no more than moderate. Mice will be monitored daily and will be killed if they deviate from normal health.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

A large part of this work will use *in vitro* tissue culture techniques, particularly to assess drug response. However, to understand how cancer cells survive and grow in the body we have to use animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Before embarking on animal studies our laboratory grows tumour tissue (from patients) in tissue culture. These *in vitro* models inform us on drug dosing for subsequent animal studies. Tumour tissue (from mice) are similarly tested (in vitro) for their response to treatment, thus reducing the number of animals used unnecessarily. Only mice with responsive tumours are used for further testing.

Refinement

Explain the choice of animals 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.

Refinement

Mice are unique in their ability to accurately model prostate cancers. The mice we use lack an immune system, thus the human tumour cells will survive and grow. The

tumours may grow to a size big enough to cause minor discomfort, in which case we will give pain-relief. The effect of chemotherapies will be closely monitored and to minimise animal suffering, mice will be killed if they show signs of distress. For all studies, dose levels will be guided from prior *in vitro* studies to minimize toxicity

PROJECT 116

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The Neuropsychopathology of Trypanosomiasis Infection
Key Words	Infection, Trypanosome, Cognition
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The parasite *Trypanosoma bruce*i is transmitted by the bite of the Tsetse fly and causes the fatal disease African sleeping sickness in humans and the related wasting disease Nagana in cattle. The human disease has two stages; during the first stage the parasite multiplies in the blood causing inflammation and fever, and in the second stage it invades the brain causing psychotic symptoms and perturbation of the sleep-wake cycle, followed by coma and death. Even where treatment is successful, many patients cured of the second stage experience continued sleep disturbance, difficulty walking and/or psychotic symptoms.

This project aims to explore the origin of the psychotic symptoms and perturbation of the sleep-wake cycle in the second stage infection, and to link them to the biochemistry of the parasite.

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

This project will lead to better understanding of the host-parasite interaction, and the mechanism that results in the observed clinical symptoms. Understanding the origin of the clinical symptoms will help underpin the development of therapeutic interventions to alleviate these symptoms, and may lead to better fundamental understanding of brain function.

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

We expect to use 225 mice over a five year period to enable us to detect changes in the symptoms of infection with genetically modified parasites. We use mice as there

is an established mouse model of stage two African sleeping sickness, and mice are the lowest sentient species suitable for behavioral studies.

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?

Mice will be anesthetised and injected with Trypanosoma brucei, and the progression of the disease monitored by taking small amounts of blood to observe and count the parasites. The activity of the animals will be observed and they will periodically perform behavioral tests. Some animals may show signs of illness during the later stages of the experiment such as poor coat condition, hunching or reduced activity and altered behavior. All animals will be humanely sacrificed if effects such as shivering or complete inactivity are observed. It is possible that some animals will die as a result of the infection, but daily monitoring of animals showing any symptoms is used to reduce this possibility. Some animals will be injected with tracer substances to allow brain imaging. At the end of all experiment the animals will be humanely sacrificed, typically under anesthetic. Post-Morten examination of tissues and fluids will be conducted.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We have to use animal experiments to find out how the parasite interacts with its mammalian host, where the parasite goes in the brain, and the effect this has on the host’s behaviour. This infomation cannot be obtained from cell culture or by using non-protected animal alternatives, as only the animal host is capable of displaying the changes in behaviour that are charactersistic of the clinical infection in humans.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We use the minimum number of animals required by monitoring the behavior of each animal in several different ways, and use only enough animals to gain significant results. We make use of sensitive analytical techniques to minimise the amount of animal material required for analysis.

Refinement

Explain the choice of animals 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.

Refinement

We use the mouse model because it is are predictable, well established and have made possible much of the work already done on trypanosomiasis. Mice are the lowest sentient species suitable for behavioural studies. Whenever possible, we use tissue culture-derived parasites in our experiments and only transfer to animal work when it is needed to find out how the parasite interacts with its host.

We minimise the suffering caused to the experimental animals by administering anaesthetic prior to injections. We monitor animals daily, and more often in the stages of infection when clinical signs are likely, and animals showing distress are humanely killed to prevent suffering.

PROJECT 117

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Therapies and model development for high risk pathogens
Key Words	Medical countermeasures, protection, infectious diseases
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aim of this project is to investigate drugs and/or vaccines for a range of highly infectious pathogens, in an animal model that is very similar to human disease/outcome. Small animal models have been used to understand these diseases, and vaccines/drugs have been tested for effectiveness. The most promising drugs/vaccines then need to be tested extensively and become licensed for use. Generally, this involves a number of clinical trials with the data being presented to a regulatory body. However, for the pathogens covered in this license, the diseases do not naturally occur predictably or in large enough numbers within human populations. Therefore, traditional clinical trials are not possible. The Food and Drug Administration (FDA) has introduced “The Animal Rule”, which allows information generated in animals, that are very similar to humans (especially nonhuman primates), to be used for licensing products. The work in this license will be performed to gather the information necessary to get drugs/vaccines licensed for human use.

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 benefit will be the advancement of medical countermeasures for human use. Additionally, this work will improve the knowledge of the disease causing process.

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

The only animal species to be used under this PPL is the common marmoset (Callithrix jacchus) with a maximum of 600 animals being used over a five year period.

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

The work on this licence involves understanding the disease process for a range of high risk pathogens. To gain an initial understanding of the disease, pilot studies will be performed with a small number of animals which are likely to get ill and may die from the disease. However, based on previous experience and the use of remote monitoring, timely intervention is highly likely which will avoid the vast majority of these animals dying. Usually sick animals will have a high, prolonged fever, tiredness and reluctance to move. These animals will then experience a severe severity. The work will move to a less severe protocol (moderate) where work will be undertaken to look at the effects of changing the dose and working out the effect of the disease at different times following infection. Information generated will help define humane endpoints, so animals can be euthanised to further reduce any suffering. In parallel, the effects of therapies such as antibiotics, or vaccines will be assessed in animals at non-toxic doses. All these animals will experience a mild effect, but a few animals (~10%) may experience a moderate effect as it may be necessary to surgically implant a device into the animals to deliver the drug. These animals may remain alive at the end of the study for re-use in other studies. Finally, the drugs and/or vaccines will be tested to see if they work against the disease. The majority of these animals (>75%) will only experience a mild to moderate suffering due to the disease. However, on some occasions the drug and/or vaccine may not work and the animals may experience a severe effect. To minimise the impact, pilot studies will be performed using a small number of animals before using larger groups if the result is promising.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

To determine the effectiveness of the therapy, including the complex interactions essential for the production of protective immunity for vaccine, there is a need for a complete physiological system. Initial studies will have been performed in vitro and in rodents and marmosets will only be used as the ‘last step to man’.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Pilot studies will be performed in 2 or 4 animals to determine the appropriate number of animals to be used in future studies, in consultation with in-house statisticians. The maximum amount of information will be obtained from each animal and samples will be stored for future exploitation and for use in other programmes e.g. diagnostic. Animals may be re-used where appropriate. All data will be written up for publication in the open literature and shared at appropriate scientific forums to reduce duplication of experiments. When multiple therapies are being assessed, control animals will be shared. The use of imaging may be investigated to determine whether it is possible to reduce the overall number of animals in model development and efficacy studies.

Refinement

Explain the choice of animals 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.

Refinement

Infected animals will be monitored using remote telemetry (video and temperature monitoring). Humane endpoints (i.e. a suitable scientific endpoint that will minimise any potential suffering of the animal) will be used. Additionally, where appropriate animals will be familiarised to the procedure (e.g. handling, environment, personnel) or trained using placebos e.g. milkshake in a syringe for oral administration. Animals will be habituated to personnel doing the work before it starts. Appropriate environmental enrichment will be provided and include a selection of the following, detachable veranda to allow both visual and vocal interactions with other marmosets, puzzle feeders, a tub to allow deep litter foraging, Tupperware boxes for sitting in, etc. Familiar items such as sleeping buckets will be transferred with animals when it moves locations to ensure they have a familiar object in the new environment. The use of imaging will be investigated to determine whether studies can be refined by collecting repeat data from fewer animals, giving better scientific data. To allow safe handling and to keep the stress of capture to a minimum a soft netted restraining mechanism has been incorporated into the containment cage design. Positive reinforcement will be used to increase compliance of animals e.g. to accept oral drugs etc.

PROJECT 118

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Evaluation of new treatments for polycystic kidney disease
Key Words	ADPKD, polycystic
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall aim of the project is to identify novel therapeutic strategies to treat Polycystic kidney disease. Compounds are tested for their ability to reverse or slow down kidney cyst formation and improve kidney function and for adverse events to be minimal in order to provide significant improvement in the quality of life compared to current 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)?

Polycystic kidney disease is a debilitating disorder characterised by progressive kidney cyst formation leading to end stage renal failure. It affects about 70000 adults and children in the UK and accounts for 1 in 8 people in need for a kidney transplant. This work is expected to provide new information on mechanisms regulating kidney cyst formation, which will be used to identify new therapies with better efficacy and fewer side effects.

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

18000 mice 2000 rats both 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 levels of severity? What will happen to the animals at the end?

Kidney cyst growth and progressive related perturbations in function will be induced in rodents by subcutaneous or orthotopic implantation of cystic human cells or by genetic deletion of proteins known to cause the disease in humans. The majority of studies described in this license are well tolerated by rodents and those models are

well described in the literature. It is not expected that serious adverse effects will occur but any side effects are likely to involve bodyweight loss and deterioration in clinical signs. Any animals exhibiting such signs will be removed humanely from the study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Polycystic kidney animal models are required to assess the effect of a test compound on kidney cyst formation and global renal function (efficacy)

Cell and ex-vivo assays can give a good indication of the potential ability of a compound to modulate the formation of cysts but they cannot fully predict in vivo efficacy on global renal function or disease progression. In vivo models are therefore an absolute necessity to relate in vitro data to efficacy in order to predict a potential clinical benefit. In addition, the PK/PD relationship, driven by distribution, metabolism and elimination, cannot be accurately modelled in vitro.

Finally, proven in vivo efficacy data is a prerequisite of the regulatory bodies who have the authority to approve or reject a new drug application

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Protocols covered by this project licence application are designed to use the minimum number of animals possible.
Tolerability studies are performed with small groups of animals in order to establish the maximum tolerated dose and suitability of dosing regimen prior to larger efficacy studies. Only then, can the more complex in vivo efficacy studies commence in the knowledge that the animals are likely to tolerate the compound.

Minimum group sizes for efficacy studies will be calculated using power analysis and will incorporate consultation with a statistician.

The use of non invasive techniques to repetitively record both cyst/kidney volume and glomerular filtration rate will avoid unnecessary sacrifice and enhance the amount of mechanistic data obtained in a single animal, therefore decreasing the number of animals necessary for each particular study.

Refinement

Explain the choice of animals 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.

Refinement

It is well documented that deletion of either polycystin-1 or polycystin-2 proteins in mice leads to progressive development of kidney cysts and loss of kidney function, which reflect the pathology and biochemical changes associated with the human disease. These models are based on the gene mutations that have been shown to cause ADPKD in human patients, therefore improving the likelihood of translation from efficacy observed in these models to a clinical benefit. Studies in rodents deliver robust, reproducible data and so it is often unnecessary to evaluate efficacy of new compounds in higher species.

The project uses techniques that can also be used in patients during clinical trials such as total kidney volume and glomerular filtration rate. These techniques will provide efficacy data on the key symptoms of the disease and key information on the mechanism of action of the compound tested that will be directly translatable to the clinical situation.

In addition to tolerability studies, pilot studies may be conducted in a small number of animals in order to refine the parameters and methodology for ensuing studies. These are intended to define the risk/benefit ratio of each procedure to generate statistically significant data whilst causing the least adverse effects.

Finally, longitudinal non-invasive imaging and/or measure of specific urine markers will be used to assess disease progression and will be correlated to the general physical condition of the animal in order to define accurate, quantitative humane endpoints.

PROJECT 119

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Generation and breeding of genetically altered rodents
Key Words	Transgenic,, generation,, mouse,, breeding
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To generate and breed genetically altered animals to be used in scientific research to help understand mechanisms of cancer. Under the authority of this licence, new mouse models will be generated to satisfied the scientific demand of the research groups.

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)?

Genetically altered mice are valuable animal models that contribute to the elucidation of a wide range of biological processes and diseases like cancer. Although in vitro approaches provide critical data, the use animal models is essential to understand the very complex scenario of this disease.

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

We will use only mice and anticipate the use of 9,250 mice over the five years. Routine breeding once the new genetically altered mouse has been produced will be transferred to a separate “Breeding Licence”

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

Genetically altered animals will be created under this licence in order to understand further different processes associate with cancer. The various steps involved will be: 1: Injection of hormone to increase egg production in female mice. 2: Female mice may have embryos implanted. 3: Vasectomy of male mice to allow them to be used to make females have phantom pregnancies and make them ready for receiving the

test-tube generated embryos. New mouse strains will be created by manipulation of either the embryo or pre-embryo using standard gene manipulation methods. Each new strain generated will have a very well described, expected profile. However animals will be monitored for unpredicted adverse effects and profiles will be monitored. Surgical Procedures will be performed under anaesthesia and using pain relief and following asceptic methods to minimise risk of post-surgical complications. Anaesthesia will be carefully and regularly monitored to ensure that an adequate depth is maintained throughout any surgical procedure. Mice will be monitored regularly for their health status throughout all procedures. All procedures will be undertaken by trained, competent people.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

New technologies are improving the field of animal transgenesis and they will allow the generation of new mouse models to be applied in biomedical research. The different animal models generated will integrate the complete range of molecular, cellular, physiological and behavioural interactions necessary to fully understand how genetic modifications result in normal or abnormal processes, focusing on cancer.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To avoid generation of already existant genetically altered mice, all databases and cryopreservation banks will be interrogated initially to avoid duplication.

Breeding programmes will be agreed in advance and regular reviewed to optimally meet anticipated demand. Breeding programmes will be optimised wherever possible to produce only the required genotype.

Freezing of eggs / embryos and sperm will be carried as routine. Archiving of lines will avoid wastage from the need to maintain colonies by continuous breeding.

Refinement

Explain the choice of animals 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.

Refinement

Researchers have studied laboratory mice as models of human cancer for many years. Their use as cancer models has provided exceptional insight into the biology and genetics of human cancers. There are standard protocols, methods and reagents used that have been optimised for manipulation of genes in this species and their acknowledged benefits for use.

The mice will be cared for by dedicated, experienced animal technologists who have the expertise and skills required to breed mice. Welfare problems that may occur at an early stage will be monitored carefully to determine appropriate end points in consultation experienced animal husbandry technician and veterinary surgeons.

PROJECT 120

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Role of experience in synaptic translation in vivo
Key Words	Sleep, circadian, plasticity, translation regulation, synapses
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Sleep helps us integrate new information from the environment in the brain on a daily basis. But how sleep achieves this function, in particular at the level of molecules, is not well understood. An important molecular step that promotes the stabilisation of new information in the brain is the production of new proteins (i.e., protein synthesis). New proteins will provide the building blocks necessary for the restructuration of communication between brain cells. Work in the past 20 years has identified that protein synthesis can occur directly at the synapses – the site of brain cell communication. Protein synthesis will thus allow to modify and stabilise each synapse individually in response to new experiences during wakefulness. Despite considerable evidence that sleep is a preferred time for protein production compared to wakefulness, the way how sleep regulates protein synthesis at synapses has never been investigated.

This project aims to address the **hypothesis that sleep benefits brain plasticity (i.e. integration of new information into existing brain circuits) during development and adulthood by primarily regulating protein synthesis specifically at synapses**.

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)?

Protein synthesis at synapses is at the basis of our general cognitive abilities. This is because it is involved in the creation of synapses during development and their constant modification (i.e. experience, learning) during adulthood. Dysregulation of protein production at synapses are known to be responsible for several neurodevelopmental disorders (e.g. autism spectrum disorders). Since sleep

amounts are highest during early life and lowest during ageing, insights into the role of sleep in synaptic protein synthesis will provide an important starting point for future investigations at both the fundamental and the clinical level.

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

This project will be conducted in rodents (mice and rats). Given that sleep amounts, as well as cognitive performances, vary across lifespan, we propose to compare results from 3 different groups: young, adult and aged rodents. We expect to use an overall amount of 850 animals 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 levels of severity? What will happen to the animals at the end?

The interventions involved in our experiments include breeding of genetically modified mice, monitoring of brain activity using electroencephalography (EEG) and behavioural manipulations. At the end of each experiments, animal will be humanely killed (according to the ASPA Code of Practice) for tissue collections necessary for our molecular measures. Breeding will not generate any adverse effect. Behavioural manipulations include short term sleep deprivation and housing in an enriched environment. The amount of sleep deprivation has been carefully chosen to be the minimum required (6 hrs) to test our hypothesis and housing in an enriched environment is known to increase the animal's well-being. The level of severity due to the surgical procedure for EEG implantation is expected to be moderate. This surgery is a standard procedure performed for more than 40 yrs in rodents and involves implantation of electrical wires into small holes made in the skull of the animals. This procedure will be conducted under anaesthesia and pain will be minimised with the use of perioperative analgesia. Animals will be carefully checked at regular intervals following the surgery and if the animal's welfare is in any way compromised, advice from the in-house veterinarian will be sought.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Sleep is a very complex state, involving changes in the entire organism and many physiological parameters (e.g., temperature, hormone levels, type of brain activity). The interaction of all those factors is necessary to fully express sleep's beneficial role on cognitive function. It is therefore necessary to study the molecular mechanisms underlying the role of sleep in brain plasticity in the **intact organism** (i.e., *in vivo)*. Simplified models (e.g., i*n vitro) are* inherently associate with disrupted

neuronal connections and can therefore not adequately reproduce the complete array of biological interactions necessary to fully understand the basic mechanisms investigated in this project. Other alternative approaches, such as mathematical models, are limited to mimic the complex interaction that exists between the brain and our environment, **which is a critical process studied under this project**. Thus, there is no feasible alternative that would entirely replace the use of a living animal and would allow the project objectives to be met.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

In addition to the use of inbred rodent strains to reduce inter-individual variability in our molecular and physiological measures, our choice of paradigms and methods contributes to Reduction:

Synapses are very small structures. Isolation of molecules from synapses thus requires overall more starting material compared to traditional whole cell molecular extract. To minimize the number of animals used, we will favour rats over mice for tissue collections since the former have a significantly larger brain.

The use of different transgenic mouse lines will allow to optimise the specificity our molecular measures and to identify specific types of brain activity underlying those changes.

To reduce the number of experimental groups, we will use telemetry that allows the recordings of several parameters simultaneously (EEG, motor activity and core body temperature). We will also implement efficient experimental design to apply statistical analysis that will assess simultaneously the contribution of several factors (e.g., “age”, “type of behaviour”, “sleep vs. wakefulness”) on our molecular measures without increasing the number of animals for each experiment. Finally, the number of animals will be carefully adjusted to be the minimum required to achieve the objectives using advice from our in-house statistician.

Refinement

Explain the choice of animals 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.

Refinement

Sleep states and EEGs, similar to those known to be important for cognitive function in humans, are only seen in mammals and can therefore not be studied in lower organisms or simplified systems (i.e. in vitro). In this context, the combination of a

wide range of molecular and genetic tools, as well as available data on the relation between sleep and brain plasticity, make rodents our model of choice.

All molecular methods and behavioural paradigms we propose to use have been developed and validated in the rodent model. This will not only ensures a high success rate for our proposed experiments but will also provide useful information for comparison with fundamental and clinical published data on the subject.

Finally, our research group has extensive experience in animal behaviour and EEG surgery and monitoring (> 10 years). The telemetry system has been used in-house for several years and all procedures to minimise pain and distress to the animal have been refined over the years following the advice from the in house veterinarian.

PROJECT 121

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Understanding gene – environment interaction in inflammatory bowel disease
Key Words	Inflammatory bowel disease, Crohn's disease, arthritis, Infection, Tumours
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Crohn's disease and ulcerative colitis are the two major forms of inflammatory bowel disease. These are chronic, debilitating diseases that usually occur early in life and lead to severe inflammation of the intestinal tract. These diseases ruin lives, and can also lead to cancer. The UK is amongst the countries with the highest risk for this disease, it is estimated that ~1 in 200 individuals is affected. Risk for these diseases runs in families, and the genes responsible for this risk have been largely discovered over the last decade. However, the genes in themselves do not cause disease, but it requires other – yet unknown – factors that trigger disease. Unfortunately, even the function of most of the risk genes and how they would lead to disease, remain mostly unknown. Here we aim to study the function of important risk genes to discover the 'pathways' and cells in the body that are engaged that lead to disease. We will also aim to use this knowledge to identify and study environmental factors that may trigger disease in an individual who carries 'risk genes'. In our intestine, we carry an enormous number of bacteria and other microbes, and they seem to be the target, but possibly also a trigger, of a misguided immune response that is typical of inflammatory bowel disease. We will hence study the role of the microbiota and how this affects the triggering of disease.

In summary, we want to understand how IBD risk genes function, and how they interact with environmental triggers. This will not only increase our understanding of the mechanisms that cause disease, but may identify novel targets for treatment that could subsequently be developed into new medicines.

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

We hope that our investigations will identify new treatment targets for inflammatory bowel disease, and possibly also for other immune-mediated diseases. We hope to begin to understand what triggers may set off intestinal inflammation. This is very important as this disease has become way more common in the Western World over the last decades, and is now also picking up speed in other parts of the world such as Asia.

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

Over 5 years, we will study ~24000 mice. This amounts to 1.3 mouse per day per researcher. We have a strong track record of major discoveries in this field over > 15 years, all on the background of adhering to best practice and highest ethical standards. This includes experimental design principles where we apply highest standards including random allocation to experimental groups, and blinded analysis – all meant to avoid bias introduced by investigators.

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

The vast majority of genetically-altered mouse lines we study develop entirely normally. They will typically undergo a tissue biopsy from the ear to determine their genotype, and for a large number of animals this will be the only intervention and no adverse effects are expected from that. Mice may be fed specific diets (e.g. high-fat diets), or they may receive antibiotics to modulate the bacterial flora in their intestine – interventions that are typically well tolerated and only few adverse effects would be expected that we will monitor for (e.g. diarrhoea). In a small proportion of mice we will induce colitis, infection, arthritis, or tumours. This can be associated with diarrhoea, weight loss, and compromised overall well-being. No more than 10% of animals studied in this project license are expected to show moderate clinical signs such as piloerection. Very rarely the severity of these signs may be such that the humane end points may be reached. Animals are monitored on a regular basis to detect any sign of distress or suffering. Analgesic agents will be administered as required. In the event of complications, or at the end of the experiment, animals will be killed by a schedule 1 method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The number of mice for this project may seem relatively high, although it is ~1.4 mouse per day per researcher. This is due to the lack of reliable in vitro models that

capture the complexity of the intestinal tract: there is a myriad of microbes in faeces, and a thin layer of mucus and cells that separates them from the body's intestinal cells – which, for example, contain the body's largest accumulation of immune cells. A pathologic immune response to these microbes is a hallmark of inflammatory bowel disease. There are absolutely no *in vitro* models available that would even remotely capture the complexity of the organ and therefore we have to rely on mouse models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We are using a wide array of sophisticated *in vitro* experiments, including various types of cells and cell lines, and all sorts of molecular techniques to predict the establish biological mechanisms. This allows us to make predictions and to prioritise those mechanisms that need to be studied in mice *in vivo*.

When designing experiments, we perform statistical analysis to determine the minimum number of mice that are required to perform an informative experiment. We also always aim to maximise the information we can gather from every single mouse.

Refinement

Explain the choice of animals 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.

Refinement

Animals are housed according to the best recommendations in an appropriate and enriched environment. By performing pilot studies and choosing well-established protocols based on extensive previous experience, we minimise the unknown effects on the mice and hence pain, distress and suffering. We very frequently monitor animal behaviour and well-being to detect any upcoming problem at an early stage.

For one of the most important genetic risk factor of inflammatory bowel disease (which is carried by ~two thirds of all patients), over the last years we have developed a model of small intestinal inflammation that spontaneously develops, and which is not associated with any clinical disease or suffering of the animal. It is purely visible under the microscope. This is a major advance, and we have validated that this closely resembles human Crohn's disease.

Amendment 2017 to allow animals receiving infectious agents through the nose to be under anaesthetic to enable safe and successful administration of agents without

causing undue stress to the animal. Anaesthetics are expected to have no adverse effects on the animals as they will typically be under anesthesia for only few minutes.

PROJECT 122

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Breeding and maintenance of genetically altered animals (GA) and GA mouse production and re-derivation
Key Words	Transgenic, conditional expression, cancer
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The genes which make up human tissue (the human genome) have been identified and the sequence in which they appear within the DNA material of human cells is now known. Similarly the mouse genome has also been sequenced. However, the function of many genes is not known or is not fully understood, either individually or in the ways they interact to produce their intended effects, or how the process goes 'wrong' in disease. To better understand normal physiological processes and abnormal disease processes requires, when necessary and justified, the use of whole animal models.

This project will breed and maintain rodents with genetic alterations (Genetically Altered Animals [GAA's]) and supply them for research by our own research teams into fundamental molecular and cellular functions and disease processes in the fields of biology and medical science. At this institution they are used to study a variety of conditions including cancer, neurology, musculoskeletal disease and ophthalmology as well as to gain further knowledge of the fundamental processes within human cells. In many cases this has led not only to new knowledge but to new treatments for human diseases. We will also provide animals, embryos or sperm when to other institutions, thus avoiding the need to duplicate imports of animals from elsewhere The licence allow us to create new GA lines, maintain frozen embryo stocks of key strains when not required. Perform embryo transfer re-derivation as a method of removing pathogens and establishing clean stocks of mice. Allow transportation of embryos and sperm to and from other institutions.

Animals will be bred by qualified animal technologists using standard breeding and husbandry protocols and the highest levels of animal health and welfare will be maintained at all times.

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 many cases this has led not only to new knowledge but to new treatments for human diseases. We will also provide when requested animals to other institutions, thus avoiding the need to duplicate imports of animals from elsewhere. This will allow us to maintain frozen embryo stocks of key strains when not required, thus reducing the number of mice bred representing a major refinement of current procedure. In addition, embryo transfer also has some advantages over hysterectomy re-derivation as a method of removing pathogens and establishing clean stocks of mice. Transportation of embryos rather than live animals is a good alternative as it avoids problems with airlines regarding shipment and is a key refinement as it avoids stress to the animals.

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

Over five years we expect to use up to 96,400 mice to provide a complete service from creation, to breeding and maintenance to transfer to end users with a justified and licensed programme of work.

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?

In the majority of mice the severity is expected to be subthreshold as animals do not show any phenotype and are indistinguishable from wild type mice. Moderate models will be monitored closely and humane endpoints adhered to avoid adverse effects. Animals will either be supplied to other licences for experimental use or be used as of the breeding process. Animals will be culled by a schedule 1 method at their end of their economic breeding life. A miniorty (10%) of moderate strains may exhibit more harmful phenotypes such as manifestation of spontaneous tumours which are used for human models of disease. At this point they will be transferred to a Project licence authorising their use in cancer studies. Animals held under this breeding project licence that exhibit a moderate phenotype and will not be transferred to an experimental licence, will be humanely culled in accordance with their humane endpoint, for example weight loss of 20%. Animals will be managed by not allowing them to reach the age where harmful phenotypes are expressed and humane endpoints are carefully adhered to. Some animals not exhibiting any adverse phenotype will be used to continue breeding. At the end of their breeding life animals will be humanely killed. Some animals used for the creation of new strains and rederivation will undergo surgical procedures such as vasectomy or surgical implantation of embryos via the abdomen (laparotomy).

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

While many of the research projects will involve the use of in-vitro systems such as cell culture, human tissue assays, computer modelling to complement the animal work. The use of genetically modified mice is essential to study gene function during development and in disease as there is no non-animal alternative available due to an inability to reconstruct complex biological systems of cell or tissue culture.

In-vitro assays cannot adequately model the complete array of molecular, cellular, physiological and behavioural interactions necessary to fully understand how genetic modifications result in normal or abnormal processes.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The strain used for generating a new colony will be carefully considered to avoid producing unwanted mice. Animals will only be bred if a user requirement has been established, and the breeding programme will be subject to regular review to optimally meet anticipated demand. Spare animals will be made available for use on other scientific projects and Wild Type animals produced by heterozygous mating's will be used as controls where possible.

Breeding will be optimised, wherever possible, to produce only the genotype required e.g. Homozygous breeding pairs.

Cryopreservation of gametes and embryos to archive lines will avoid wastage from the need to maintain colonies by continuous breeding.

Breeding programmes are optimised and managed as efficiently as possible to minimise wastage and to align demand with supply. Various strategies are used to achieve this and breeding programmes are regularly reviewed. Where excess animal production cannot be avoided, animals are made available for tissue use.

Refinement

Explain the choice of animals 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.

Refinement

Mice are universally used for work involving genetic alterations. The standard protocols, methods and reagents have been optimised for this species and there are

acknowledged benefits from their use. Best practice will be followed where practically possible with reference to the following:

Home Office Efficient Breeding of GA animals assessment framework http://org.uib.no/dyreavd/Documents/GAA%20tool.pdf

Genetically altered mice NCRs https://www.nc3rs.org.uk/GAmice

NC3Rs Blood sampling

https://www.nc3rs.org.uk/3rs-resources/blood-samplingGA mouse welfare

In this licence the surgical technique for performing vasectomy has been refined so the scrotal approach is used and which is less invasive than the laparotomy approach.

PROJECT 123

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Understanding how to exploit the oxygen sensing pathway for therapeutic benefit
Key Words	Oxygen, Immunity, Cancer, Therapy
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Oxygen and the ability to sense oxygen is essential for our survival. Cells throughout our bodies continuously monitor the amount of oxygen available to them through a system called Hypoxia-inducible factor (HIF). When oxygen levels are low (a condition called hypoxia), HIF is activated enabling cells and tissues to adapt to low oxygen. The importance of this oxygen-sensing pathway is evident in that it operates in all cells and has many different functions. As a result, the pathway plays a role in a range of normal and disease settings including ischemia, stroke, heart attacks, energy homeostasis, immunity and cancer. This licence will focus on two of these areas, immunity and cancer.

The overall aim is to use genetic and pharmacological approaches in mice to understand the consequences of manipulating HIF. We anticipate that pharmacologic manipulation of the HIF pathway will be useful in a range of immunological diseases and cancers.

The role of the HIF system in certain specialised immune cells is largely unknown. The immune system produces proteins known as antibodies that protect our bodies against infection. The immune system also produces memory cells and is the reason why vaccines work. The HIF system has major effects on certain aspects of immunity but we have just begun to scrape the surface. We wish to comprehensively understand the role of HIF in immunity. This is important since the hypoxia pathway can be targeted with drugs and so there is capacity to use these drugs as novel treatments to enhance responses to vaccination or treat autoimmune diseases, transplant rejection and cancer.

The second major area we will focus on is cancer. Hypoxic cancer cells are aggressive, resistant to treatment and contribute to cancer spread. We will study how HIF contributes to these processes and how it could be targeted with drugs as a novel treatment for cancer.

The “oxygen-sensing” mechanism that regulates HIF centres on a particular family of enzymes. Drugs that block the function of these enzymes mean that HIF can be activated in the presence of oxygen – i.e. in situations where it would normally be destroyed. There have been recent advances of these drugs in clinical trials. In this project we will study the effects of inhibiting these enzymes and HIF. We need to find out which enzyme(s) it is best to inhibit, which disease(s) this will be helpful in, what stage of the disease it will work at, and what the side effects will be.

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

We are focusing on how HIF influences immunity, immunological diseases and cancer. Ultimately, by identifying and understanding its role, it may be possible to develop novel treatments, since the HIF system is pharmacologically tractable. We anticipate that pharmacologic manipulation of the HIF pathway will be useful in these clinical settings. This project in mice will help us to select the right pharmacological target(s) to achieve this, guide the choice of the clinical circumstances in which this is most likely to be useful, and indicate what side effects are likely to occur. Importantly, we know that both the immune system and HIF system are very similar in mice and humans, making mice an excellent translational model of our findings to the human setting. The genetically altered mice developed will be made available to other scientists working in the field. Findings will be made available to other scientists through publication in peer-reviewed journals and presentations at scientific meetings. This will advance our scientific knowledge and therapeutic application of this research field faster.

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

Over the 5 year course of the licence we expect to use an estimated 8500 mice.

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

The majority of mice will be genetically altered (GA) mice used for breeding and harvesting tissues for analysing cells cultured in the lab. Most GA mice will be healthy and show no harmful effects (sub-threshold severity). Since most of our GA mice will have genetic modifications in the immune system, mice are maintained in a ‘barrier’ environment in individually ventilated cages to protect them from infection and ensure that any effect on the immune system does not compromise the health of the mice. A very small number of GA mice may be found with physical signs of ill

health. These mice will be closely monitored and mice showing signs of discomfort such as reduced movement and weight loss will be humanely killed. Additionally a very small number of GA mice may be found dead. This is because the onset of clinical signs is rapid such that mice may not be found showing clinical signs of ill health even when inspected 2-3 times a day, and so it has not been always been possible to humanely killed the animals at an earlier time-point. The majority of mice that undergo a regulated procedure will only reach mild severity, meaning that mice will experience no more than transient discomfort and no lasting harm. In some circumstances, mice exhibiting adverse effects may undergo a procedure and enter moderate severity. Here the health status will be closely monitored and recorded. Mice exhibiting signs of distress will be humanely killed. Experiments to test the effect of altering the HIF system in cancer involve injecting cells under the skin of anaesthetised mice, mice recover and the cells grow as a tumour. The tumours will be monitored carefully and if they reach a certain size, or are affecting the animal's health the mouse will be humanely killed. Mice dosed with drugs or other agents such as cell labelling dyes, in the drinking water are expected to enter subthreshold severity. Mice dosed with substances in the drinking water or diet that alter their genetic make-up are expected to tolerate this well, some mice may not eat or drink as much. Other procedures include injections, blood sampling, and immunisations that mimic human vaccines. Mice receiving these procedures are expected to not show minimal or no signs of distress and enter mild severity. Some mice infected with a mild virus may develop mild flu-like symptoms which last no more than 24 hours. Some mice will receive a dose of radiation to enable bone marrow transplants of immune cells. This may cause some mice may to feel unwell with diarrhoea as a result of irradiation. However, we have not seen this thus far in our studies. Some mice will live in low oxygen environments for up to 3 weeks (moderate severity), which will cause them to not eat or drink as much as normal for a short period (2-4 days) but then acclimatise back to normal eating and drinking behaviour. At the end of experiments, most mice will be killed by a humane 'schedule 1' method. In rare and exceptional circumstances we may use non-schedule 1 humane methods, to allow us to take large blood samples or study short-lived proteins of the HIF system.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Wherever possible, we also perform human studies, but this is not always feasible. We will minimise the number of mice used by performing as much preliminary work as possible in cell cultures or on live cells isolated from mice. Our approach to combine human and animal data with state-of-the-art computational methodology

allows us to explore HIF in certain clinical settings that are closely translatable to humans. These studies allow us to optimise and focus our studies.

Work proposed under the PPL can only be undertaken in an intact organism in which the complex interplay between different cell types and in different organ systems can occur. We have previously used C. elegans (a worm) very effectively but we cannot use this to study adaptive mammalian immunity. For studies involving the use of cancer therapeutics, drugs are first tested extensively in cultured cancer cells in the lab before being considered for use in mice. Only those showing a significant therapeutic effect in cancer cells and not normal cells are considered for use in animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of mice bred are kept to a minimum. Existing GA mice are bred when possible to avoid generating new mice. Complicated breeding strategies are continuously monitored and reviewed. The strain administration is centralised to ensure coordination of different studies and prevent experimental duplication. The information obtained from each mouse is maximised by measuring as many variables as possible.

Experiments are designed in keeping with the ethos of the three R's: We use blinding and randomisation widely during our experiments to avoid biases and use power calculations to ensure that experiments use the fewest number of animals to generate statistically powered data. We carry out pilot studies if we are planning complex dosing and/or immunisation studies that require initial validation and optimisation. These steps ensure that we generate high quality data and maximise the insights we obtain, whilst using the minimum number of mice to produce statistically valid data.

Additionally,when planning and conducting experiments we make use of the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines https://www.nc3rs.org.uk/arriveguidelines and publish in accordance to these. These very useful guidelines are intended to improve the reporting of research using animlas, maximising information published and minimising unnecessary studies. We are strong advocates of these.

Refinement

Explain the choice of animals 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.

Refinement

Parallels between the human and mouse HIF and immune systems are well understood, making mice a good model species for these studies. We perform many cell culture experiments and experiments on live cells isolated from mice to optimise and focus our studies in mice. Breeding mice that may develop a harmful phenotype will be kept to a minimum and only done when these mice are critically required to answer specific scientific questions. Dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm.

PROJECT 124

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	microRNA-based interventions against loss of muscle mass and function resulting from in utero and early post-natal protein restriction.
Key Words	Ageing, microRNAs, pre/postnatal protein restriction
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The reduction in muscle mass and strength that occur during ageing has a major impact on the quality of life of older individuals. Older people demonstrate loss of confidence in walking and reduced mobility which in turn leads to loss of independence and social isolation. These changes occur partly because we lose a large proportion of the muscle cells (called muscle fibres), but also the muscle cells that we retain are weak. It is currently unknown how muscle fibres are lost during ageing. There is considerable evidence that poor maternal nutrition leads to a number of changes in muscle of the offspring that result in reduced function. Muscle strength is also compromised in older individuals who did not grow well in early life, and studies suggest that maternal, developmental and nutritional factors are important. microRNAs are small molecules that regulate gene expression resulting in different sets of proteins being present in the cells. Through this, microRNAs regulate cell functions. It is established that most biological processes, including muscle growth and wasting and ageing are, or are likely to be, regulated by microRNAs. The levels of microRNAs in muscle and other tissues change during ageing and upon changes in diet. As microRNAs can regulate the expression of many genes, and therefore physiological processes, they are likely candidates to regulate the effects of poor diet on muscle.

We hypothesise that a reduction in protein intake during foetal and early neonatal life results in modified microRNA-target interactions in muscles of the offspring and this leads to loss of muscle mass and function which has long term effects on the number of muscle fibres and this ultimately adversely influences whether older individuals can maintain good muscle function as they age. It is not possible to directly examine this possibility in humans and this project will therefore use mouse

models. We will determine the effect of the reduced protein intake in utero, or in the early post-natal period, on muscle fibre number and muscle mass and function in adulthood and ageing and whether restoration of microRNA levels in muscle will prevent the loss of muscle mass and function associated with in utero and/or early post-natal protein restriction.

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

The research proposed is of high importance and we anticipate that the outcomes of this study will lead to a greater understanding of the role that diet plays on the processes underlying the loss of muscle mass and musculoskeletal function in older individuals and hence to the logical development of interventions to correct these processes.

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

The expected number of animals used in the experimental protocols is several hundred mice over 5 years. These will be young, adult, old WT or genetically modified mice.

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

The expected adverse effects, although very rare, may be associated with muscle weakness and resulting (sporadically) in change in movement. These will be monitored for through observing the mice daily, weighing the mice. Any significant changes or moderate changes lasting more than 3 days will result in animal cull by schedule 1 and tissue collection. At the end of the protocols, mice will be culled using schedule 1 or terminal anaesthesia.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Due to the nature and complexity of the experimental design and in particular the need to reduce protein intake during pregnancy and during lactation of the offspring it is not possible to use human tissue, therefore the proposal requires the use of in vivo studies in mice. In addition, because of the need to study the interplay between nerve and muscle cells and ageing of post-mitotic tissues, it is not feasible to use a cell culture based approach. It is not possible to imitate the innervation of muscle ex vivo. The rodent is the lowest species that demonstrates comparable nerve-muscle

interactions. Mice are the lowest vertebrate group possible in this study and the availability of genetically altered mice will provide definitive data necessary to achieve the objectives for this study. Other species have been considered but deemed unsuitable for these experiments, such as Zebrafish, drosophila or nematode. Ageing is a whole organism phenomenon. The ability to examine the effect of age on muscle structure and function is not possible in cell culture. The alternative is the use of primary culture to generate muscle from aged mice. However, this raises the problem of the lack of a suitable model to perform muscle force measurements in culture.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The proposal has been designed with the help of statistical advice to minimise the number of mice used. Calculations suggest that n=12-18 per time point will be necessary to achieve statistical significance.

Refinement

Explain the choice of animals 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.

Refinement

Most of our work will be carried out in mice. Mice are chosen for these experiments due to the similar physiology between mouse and human musculoskeletal system. Mice are the lowest vertebrate group possible in this study and the availability of genetically altered mice will provide definitive data necessary to achieve the objectives for this study. The mouse is relatively short-lived, therefore the effect of age on musculoskeletal structure and function can be fully documented over a relatively short timescale.

The intravenous, intramuscular and intraarticular injections and the dose of microRNA mimics and inhibitors have been optimised by us. We will use the lowest dose and the minimal number of injections to obtain statistically significant results based on power calculations and previous data. Pre- and postnatal protein restriction is a well-established technique, currently used in the UK. A number of check points for minimising animal suffering are included in the protocol design, including clear end points, shortest time necessary and minimal number of interventions. The applicants have considerable experience in all the techniques used.

The general experimental designs and methods of analysis of the results have been discussed with REDACTED

PROJECT 125

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanisms in renal inflammation
Key Words	Kidney, fistula, dialysis, inflammation, disease
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project aims to gain a greater understanding of the causes of and possible treatments for inflammation of the kidney – also called glomerulonephritis, a leading cause of kidney disease. Treatments for kidney disease may involve medicines along with dialysis (putting the patient's blood through an artificial kidney) or a kidney transplant. We also aim to understand the causes of stenoses in arteriovenous fistulas (AVFs) used for haemodialysis.

The causes of kidney inflammation are unclear, but appear often to be due to the body's own immune system attacking the kidneys. From this knowledge treatments have been developed that modify the immune system – although they can be effective they unfortunately make the patient more susceptible to infections and even to the development of cancer. Thebiological processes leading to stenoses and dysfunction in (AVFs) are not well understood and there are few treatments.

Although we can study naturally occurring kidney inflammation and AVFs in our patients, we are limited as to what interventions and studies we can perform. When available, we do study human tissue. But to understand the mechanisms fully we need to use animal models, and we make use of mice for this purpose.

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)?

Overall, we expect that these studies will provide important information about the mechanisms which lead to the development of kidney inflammation, and from this the development of new medicines that are more selective in their action than those currently available. In doing so, this will be of benefit to those who are stricken with this potentially life threatening condition, and hopefully prevent these patients from having to be treated by dialysis or a kidney transplant. We also aim to understand the processes causes problems in dialysis fistulas and to develop treatments for this.

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

We have determined the minimum number of animals that we will need to use overall, and we estimate that less than 1000 mice will be used in experiments on glomerulonephritis, and less than 200 mice will be used for experiments on arteriovenous fistulas, for each year of this five year project.

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?

In our studies we induce kidney inflammation in mice by injecting proteins, perhaps in combination with other agents. Mice may develop kidney failure. The adverse effects that may be seen include lethargy and hunching, and also fluid accumulation. The expected level of severity is moderate. In all studies, we will minimise the adverse effects on animals by monitoring their body weight, body condition. Any animal demonstrating signs that exceed the limits in the licence will be killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Glomerulonephritis and arteriovenous fistulas are complex biological processes that cannot be modelled in vitro and therefore animal models are needed.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of times that an experiment has to be repeated, and the required group size, are a function of the variability within and between experiments. In order to minimise variability and improve the signal to noise ratio in all these experiments, a number of steps will be taken. These include careful weight matching (in addition to age and sex matching) of animals. Care will also be taken to use controls of the appropriate genetic background in any experiments that include genetically altered mice. A sample size calculation was performed using Statmate from Graphpad with the following assumptions. Statistical test: unpaired t test, with an expected Standard Deviation of each group = 15. This is because we would expect 95% of animals to have 0-60% glomerular crescents which is within 2 SDs of 15% with a mean of 30%. Significance level (alpha) = 0.05 (two-tailed) and Power 80%. We considered a difference in crescents of 20% between groups to be biologically meaningful. A

sample size of 10 in each group is needed based on the above. Similar considerations apply to other experimental readouts, as the spread of data will be similar. A randomised block design will also be used, in combination with factorial design, to further facilitate a reduction in animal numbers.

Refinement

Explain the choice of animals 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.

Refinement

The mouse is a well-established model for experimental immunological studies, and there is large body of published data on the immune system in this animal. There are well established models glomerulonephritis and arteriovenous fistulas in mice. In respect to the immune system, the genetic organisation of the major histocompatibility complex (MHC) is well characterized in the mouse, which is similar to humans; the functions of MHC class I and class II molecules in T cell responses have been extensively studied in mouse. Also mice are the species with lowest neurophysiological sensitivity that are likely to produce satisfactory results. Genetically altered mice for which a specific gene is either deleted (knockouts) or reintroduced (transgenic) are very useful tools for the study of gene function. Use of genetically altered mice with specific genetic alterations believed to affect the immune system will allow us to identify and determine the influences of specific genes (and by consequence specific molecules) in glomerulonephritis.

Suffering will be minimised by carefully close monitoring with carefully defined endpoints for any adverse effects. Our experience with these models allows us to anticipate when mice might become unwell, and we avoid placing them in metabolic cages for urine collection as this is an additional stress. Proteinuria is assessed from spot urines as an alternative in these situations.

In the arteriovenous fistula model, only mice in whom surgery is successful will be allowed to recover.

PROJECT 126

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Avian Influenza in Wildfowl
Key Words	Avian Influenza, Wildfowl, Sampling
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The highly pathogenic strains of avian influenza are a notifiable disease in the UK as they cause widespread mortality (and serious financial loss) in domestic poultry and, occasionally, deaths in humans. Some of these strains of avian influenza can also cause deaths in the wild bird population, but in general wild birds tend to be less affected by the disease and act as vectors for its spread to domestic poultry

The aim is to define the antibodies present to avian influenza in a population of wild fowl, with a view to elucidating the reasons for observed differences of protection and influence on excretion of virus. Specifically, the aim is to determine if some birds, particularly older birds, are protected against H5N8 HPAI disease as a result of previous exposure to common, mild forms of avian influenza. This work will also allow the direct comparison with the epidemiology of the HPAI H5N1 outbreak which was studied under aREDACTED .

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

This project will increase the understanding of the mechanisms for protection wild birds against avian flu, the epidemiology of avian influenza infection in the wild bird population and the role they potentially play through acting as a reservoir of infection and hence facilitating the spread of disease to domestic poultry. This information helps government bodies such as Natural England assess the impact on countryside and environment, and the Department of the Environment, Food and Rural Affairs (DEFRA) to assess the risk to domestic poultry.

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

Up to 1120 wildfowl 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 levels of severity? What will happen to the animals at the end?

Minimal adverse effects, slight abrasions during capture in less than 0.5% of birds, some haemorrhage after of removal of the sampling needle which stops quickly in less than1% of birds.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Measurement virus secretion and antibody response to infection require the use of live birds

*Post mortem*s will be undertaken on birds that are found dead on the sampling site as an adjunct but the numbers of carcases is small and information too limited to replace the need to sample live birds.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The identification of birds by rings allows accurate targeting of birds within known age cohorts, thereby minimising the numbers sampled.

Refinement

Explain the choice of animals 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.

Refinement

All catching and handling of birds during the procedures is conducted as per British Trust for Ornithology (BTO) guidelines by handlers who are deemed competent by a BTO licence holder and under sufficient supervision by BTO licence holder.

Handling time will be kept to a minimum, approximately 10 minutes.

Cloacal/oropharyngeal swabs will be used where possible to provide additional data on viral excretion, although if a bird defecates on catching (this is very common),

non-invasive faecal samples will be taken instead. Pharyngeal will only be used if there is need to swab for high pathogenic AI.

PROJECT 127

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Tissue responses to high energy impulse loading
Key Words	Blast injury, tissue damage, damage mitigation
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Our investigations of Blast Injury have grown out of studies of injury in Iraq and Afghanistan, where the predominant cause has been the Improvised Explosive Device (IED) deployed against vehicles and also dismounted personnel. The injury pattern of in-vehicle underbody blast is complex and differs markedly from the dismounted blast environment . Other work has demonstrated that mortality from underbody blast is most commonly caused by head injury or non-compressible torso haemorrhage. The current emphasis of study of these injuries is directed at mitigation. The biomechanics of underbody torso haemorrhage (including liver laceration and aortic transection) are not understood, and are being addressed by the most recent addition to this programme of work (Protocol 3)

- to understand how tissues and organs respond to the extreme loadings typically experienced in blast, in order to guide mitigation and treatment strategies for blast related injury

to understand how visceral injuries are caused by underbody blast, and to guide mitigation strategies.

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)?

Treatments and mitigations for generalised blast injury and Traumatic Brain Injury (TBI) due to blast will be informed and developed. Treatments and mitigations for visceral injuries due to in-vehicle blast will be informed and developed. Our findings will help other researchers investigating blast injury (lung, TBI and visceral) to refine and improve their investigations and models, will provide useful information for those designing devices and strategies to protect personnel from blast injuries, and could benefit clinicians diagnosing and treating blast injury victims.

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

We will use approximately 200 rats over the duration of the licence (five 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?

Possible adverse affects are • Localised bruising and oedema may occur at the most severe blast exposures • Intubation may cause localised damage. Lung Study (protocol 2) • Animals may develop breathing difficulties as a consequence of Blast Lung. If any animal displays piloerection, hunched posture, reduced mobility, pallor, ocular or nasal discharge, or diarrhoea, analgesia will be administered or increased, and the animal will be closely monitored (twice daily). If these signs of distress persist or recur, the NVS and NACWO will be consulted and appropriate action will be taken; if after 48 hours they are not resolved by analgesia the animal will be Schedule 1 euthanised. Visceral Injury (protocol 3) In the terminal phase of the procedure the animal will be insentient throughout. Brain injury (Protocol 4) • Following TBI there may be moderate degrees of paralysis in one or more limbs, sensory loss in face and limbs, or loss of consciousness. Animals will be given access to wet mash if normal feeding/hydration is impaired. If required hand feeding will be used. Weight loss may also occur after blast injury, probably immediately after injury, and it is expected that weight would return to normal within one week. Body weight will be measured daily until it is at or above the pre-injury level. Any animal experiencing a reduction in body weight of 20% or more will be humanely killed. • Animals may lose balance and/or display impaired locomotion with the development of TBI (which is an intentional outcome of the blast treatment). This might occur immediately after injury, and can also be expected to resolve within one week. This should not be painful, and animals will be carefully monitored for any signs of distress, and, if distress is observed, analgesia will be provided immediately or increased. Any animal exhibiting persistent loss of righting reflex, ataxia or which is unresponsive to stimulation will be humanely killed

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Primary blast injury in TBI is followed by a “secondary injury” that develops hours to days later, so these injuries need to be studied and modelled *in vivo*. Likewise, evaluation of therapeutic interventions to prevent or limit secondary injury need *in vivo* studies. Computational and cadaveric models are entirely unvalidated for this work.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Previous studies in our group have determined which characteristics of shock wave result cause blast injury. Using these known blast characteristics reduces the number of animals needed to obtain sufficient data for statistical validity. The work will be carried out in accordance with the NC3Rs Arrive guidelines.

Our continued approach to research is to attempt to identify all possible confounding factors before undertaking the work, to analyse preliminary data as soon as it is gathered to check for unexpected confounding factors (and hence to adapt the experiment to account for these as early as possible), and to maintain our communication with colleagues and peers working in similar areas or models (to hear of developing lines of research and understanding as soon as possible).

Refinement

Explain the choice of animals 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.

Refinement

Rats are the smallest animal which have musculoskeletal system behaviour similar to humans: with remodelling and turnover of bone and osteonal systems like humans. Mice do not have this kind of bone behaviour. Many other systems of rats are also more similar to their human counterparts than mice, and thus rats are the smallest animal that can be used in such models.

All animals will be anaesthetised during blast imposition, and provided appropriate analgesia when recovery is undertaken.

The rats will be housed in groups with feed and water *ad libitum*, to minimise stress or distress.

The underbody loading protocol uses terminal anaesthesia, avoiding any suffering for the animals.

PROJECT 128

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	ADME and PK/PD of new substances for Drug Discovery and Development
Key Words	Pharmacokinetics, pharmacodynamics, surgery, sampling, medicine
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The work conducted under this Project Licence will evaluate substances as they are discovered and developed by Chemists as potential new medicines. They will be assessed in animals to predict what the human body does to the substance (pharmacokinetics) and whether the substance has the required effect in the human body (pharmacodynamics). To help understand how much of these potential new medicines will need to be given to humans to be effective and safe the concentrations in the blood and tissues of animals following administration will first need to be investigated. This licence will focus on developing new medicines for respiratory and immuno-inflammatory diseases such as asthma and rheumatoid arthritis. These are common conditions that affect millions of people who need more effective treatment.

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 overall benefit of this work will be to help develop new and improved medicines for patients with conditions that severely impact their quality of life e.g., preventing them from going to school and work, playing sports or even just walking up stairs. Millions of people suffer with immuno-inflammatory or respiratory diseases e.g. rheumatoid arthritis and asthma and whilst there are treatments currently available, there are some patients for which the current medication does not work therefore new, improved treatments are needed that are more effective with reduced side effects so significantly improving a patient’s quality of life. This project will provide critical data that will give researchers confidence to take the potential medicines most likely to give the greatest benefits to patients, from the chemistry laboratory, into animals and finally into human.

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

Rats and mice are the principal species used in this project licence as they are the rodent species of choice in safety testing and provide reliable pharmacological models to test the effectiveness of substances. Over the 5 year duration of this project we expect to use approximately 9750 rats and 7050 mice. Because non-rodent species are also used in safety studies there is also a requirement for pharmacokinetics in these species so we expect to use in the region of 250 Rabbits, 500 pigs and 400 dogs.

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?

Routinely healthy animals will have substances administered by various routes but most often orally or intravenously. Blood samples will be taken either with a needle and syringe from a blood vessel near the surface of the body or a cannula which has been surgically implanted into a vein. Adverse effects are not expected with these procedures with animals experiencing only mild to moderate discomfort. Non-specific signs such as discharge from the eyes and nose, changes in behaviour or posture, weight loss, and reduction in food and water intake will very rarely be seen. Post-surgery, a small amount of temporary weight loss can occur but other post-operative complications are rare e.g. suture breakage, dryness/dead skin or infection at the surgical site. Animals that have undergone surgery to implant cannula will receive antibiotics to minimise the risk of infection and pain-killers to minimise discomfort. Healthy animals may be used for more than one study but following completion of studies animals will be humanely killed. Samples of organs and tissues are often taken after death to measure the concentration of a substance in them. During procedures such as substance administration or blood sampling animals are restrained to avoid any injury to the animals and ensure success of procedures. After surgery animals may be housed in a cage on their own (most animals prefer to be housed with other animals) to avoid post-operative complications such as chewing of cannula. For some studies animals are housed on their own in special cages to collect urine and faeces for substance analysis to see how the body is removing the substance from the animal. Animals are monitored for general signs (as mentioned above) that may be due to the substances being tested however the doses administered under this project are typically low so that no adverse reactions would be expected following administration.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Much work can be conducted without using animals to reduce the number of substances from thousands to hundreds with only some of these, the most likely succeed as potential new medicines , going on to be tested in animals. These substances need to be tested in animals due to the complexity of the mammalian body and the different interactions between the body and new medicines. Understanding these interactions cannot be achieved without using the ‘whole body system’ of animals and without this information the effectiveness and safety of a new medicine would be much less understood resulting in the risk being too high to give it to humans.

The company where this project will be used continues to actively seek alternatives to animal use in research and development to reduce the number of studies requiring the use of animals, however any alternatives must be shown to be reliable, robust and accepted by government organisations that regulate new medicines for patient use globally.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Robust scientific and ethical review of all studies ensures that the number of animal studies are minimised. Statistics and study design are optimised to ensure the minimum numbers of animals are used to achieve the aims of the study and project and advice is available and taken from a qualified in-house statistician who provides dedicated support for the project. Animals may be used for more than one study, under veterinary guidance, where this is considered to be of lower impact than using another animal.

Refinement

Explain the choice of animals 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.

Refinement

The minimum volume of blood will be taken for each study and microsampling techniques will be used that require very small volumes of blood e.g. microlitres. The frequency and volumes used when administering potential new medicines will also be minimised.

All procedures, including surgery, are performed by trained and competent staff. Peri-operative care e.g., warm environment, supplementary fluids, soft food/baby

food, analgesics, aseptic technique and antibiotics, if required, all contribute to minimise the potential of surgery to have adverse effects. Durations of procedures will be kept to the minimum required for individual studies to minimise their impact on the animals. Animals are housed in cages and pens that contain bedding and environmental enrichment appropriate to that species to keep them as comfortable as possible.

PROJECT 129

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	FARM ANIMAL TREATMENT EVALUATION AND DISEASE CONTROL
Key Words	farm animal, disease
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Evaluation of veterinary medicines in farm animals, farmed birds and horses. In the UK and Europe it is necessary to demonstrate that any veterinary medicine or vaccine is safe and effective in the species (cattle, sheep, goats, pigs, horses and domestic poultry) for which it is designed before permission is granted to sell the treatment. We offer an evaluation service to companies wishing to develop veterinary medicines. Some complementary tests are conducted outside of organisms or cells and we aim to keep abreast of scientific advances to implement alternative methods as they become available. Within development projects there are normally a number of *in-vitro* studies: on occasion we conduct these types of studies and will recommend *invitro* alternatives to animal studies if these are established.

Each evaluation is a study where, for example, the animals are infected either naturally or by artificial administration with an infectious agent and then some animals are treated with the test substance normally by mouth, pour on or injection. The other animals are left untreated so that the presence of infection can be demonstrated. Standard criteria are measured so that the success of treatment can be evaluated. The data are reported in a format that can be submitted to authorities either in the UK, Europe or elsewhere for registration of the treatment. The regulatory authorities have created guidelines for the minimum numbers of animals to use in these types of studies. We consult those guidelines and then conduct our own assessments to ensure that enough animals are being used to create a statistically valid study which then avoids potentially having to repeat the study. Typically a minimum of 6 animals per treatment group are recommended.

We work to ensure that we conduct studies where we have experience and we carefully plan the details of the study to ensure that the study generates valid data hence animals do not suffer unnecessarily. We then observe the animals closely to ensure that any symptoms are monitored and managed as necessary.

Despite having many treatments available, veterinary infections continue to cause health and welfare problems in millions of animals and birds annually. This work is aimed at helping in the development process for new and often more effective treatments for farmed animals, birds and horses. These diseases have economic significance for farmers so more effective treatments will have an economic benefit for farmers and animal owners, and reduce the risk of transmission of zoonoses to 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)?

Despite having many treatments available, animal diseases (production related or otherwise) continue to cause health and welfare problems in millions of animals and birds annually. This work is aimed at helping in the development process for new and often more effective treatments for farmed animals, birds and horses and therefore improve the welfare of animals through improved treatments for parasitic diseases. These diseases have economic significance for farmers so more effective treatments will have an economic benefit for farmers and animal owners, and reduce the risk of transmission of any diseases at risk to transmission to humans.

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

Cattle - 9600 Sheep - 5500 Goats - 5000 Pigs - 4600 Horses - 945 Birds - 31700 Maximum numbers to be used over the course of the 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 levels of severity? What will happen to the animals at the end?

Typically animals would be monitored for their weight gain, and sometimes blood and dung samples are collected at intervals. Normally the animals become accustomed to being handled after the first one or two occasions. Where possible we habituate the animals beforehand to a procedure and avoid handling them where we can obtain the information in an alternative way. Normally we expect to see few, if any, abnormal signs in the animals, thus the severity is usually mild. The exception is an infection of farm animals and poultry caused by a particular group of animal disease-causing organisms (Coccidia spp.). When working with these infections we have to monitor the animals closely to make sure that we treat them or euthanase them if their symptoms appear to be approaching the upper limit of moderate, the permitted severity limit. At the end of a study the animals will be humanely euthanased or released if this is permitted.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Veterinary medicines, biologicals, feed additives and vaccines must be trialled in the target species for initial safety/tolerance and efficacy before taking forwards to larger trials. Unless otherwise recommended or if there is an established and validated tissue model available, the target animal will need to be used in these studies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Expert statisticians will be involved in study design as well as consulting guidelines set by regulatory bodies and any relevant literature to ensure that the minimum number of animals is used that are needed for a valid statistical result.

Refinement

Explain the choice of animals 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.

Refinement

The animals used are the target species for the veterinary medicines and vaccines on trial. Any models will have welfare at the centre of their design. In addition, animals on these studies will have a heightened level of observations immediately after and in the days following administration of any substance. If observations are observed that are approaching or have breached the severity limits in place, then the Establishment Licence Holder, Project Licence Holder, NACWO(s) and potentially the a member of the Animals in Science Regulation Inspection Unit will be consulted to decide the course of action. This could be either, immediate alleviating of suffering if irreversible clinical signs via euthanasia, or treatment with, for example, analgesics and anti-inflammatories in order to relive suffering.

PROJECT 130

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Neurochemistry & Behaviour in Cognitive Dysfunction
Key Words	Learning, Memory, Ageing, Neurotransmitters
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To understand the chemical brain changes which occur in the brains of people as we age, and to explain how these changes causes cognitive deficits in learning and memory.

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 potential benefit of this work would be to increase our basic understanding of what happens in the brain during learning and memory tests, and to determine what neurochemical changes occur in the brain; this will help us to identify novel targets for therapeutic intervention.

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

Rats, at different ages (adolescent, adult and aged), approximately 300 per year.

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

We expect the aged animals to show a cognitive impairment. The learning and memory tests they will perform will not have any adverse effects. The likely/expected severity of procedures is mild to moderate. At the end of the designed experiments animals will be killed humanely and tissue fully utilised to further our understanding of the neurochemical and molecular mechanisms behind any behavioural changes we have observed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In order to achieve the benefits of the proposal it is vital to study complex behaviour patterns such as cognitive performance, and this may only be studied in whole animals, which unfortunately means the use of rodents. To date there is no suitable alternative to the use of whole animals for behavioural research. There are no current computer models to mimic brain function adequately. Cell culture systems are also insufficient because they do not preserve the functional architecture of intact brain circuits. Using rat brain is the closest approximation we have to studying changes that occur in the human brain. In all instances, other methods will be considered and whole animal studies will only be used where no alternative procedure is available.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The project has been designed based on our preliminary previous work in order to minimise the number of rats needed to see a meaningful difference between the groups. Statistical power calculations have been used to find the minimum number of animals to give us a conclusive answer. If through the course of the experiments we can gain significantly relevant information with fewer animals, the study design will be changed accordingly.

By refinement of our methods (e.g. by combining behaviour with neurochemistry and/or by taking the brains from the animals following behavioural testing for post-mortem analysis) we aim to reduce the number of animals used in the overall studies, but still gather a large amount of data.

Refinement

Explain the choice of animals 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.

Refinement

Rodents are among the lowest species to display behaviours which are considered similar to humans and in which behaviour can be measured. Rats are a popular choice for much preclinical work because of the already detailed existing knowledge of their brain structure and neurochemistry. Many of the studies on which the current research program is based have been performed on rats, and therefore a wealth of information exists on which to base theories under scrutiny.

All procedures are of the minimum severity level required to produce the required effects. Anaesthesia and analgesia will be used to minimise any suffering due to surgery. All behavioural tests are mild and will not put the animals under any unnecessary stress.

PROJECT 131

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

Project Title	Motor control networks in health and disease
Key Words	Spinal cord, Motor Neuron Disease, Spinal cord injury, Movement
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Simple movements such as breathing and walking are controlled by networks of neurons within the brainstem and spinal cord. These networks can function in the absence of input from the brain or sensory systems. In other words, the brainstem and spinal cord contains all that is required to control breathing and walking. At present, the networks of neurons which control these rhythmic movements remain poorly understood. If we are to develop treatments for injury and disease affecting the brainstem and spinal cord, it is critical that we understand a lot more about how the nervous system controls movement.

The first goal of this project is therefore to advance our understanding of motor control networks by identifying specific populations of cells which make up these networks and determining their roles in the control of breathing and walking. The second goal of this project is to reveal disease mechanisms which lead to the death of motoneurons, which provide signals to muscles to make them contract, in devastating diseases such as Motor Neurone Disease (MND). MND is an incurable disease which develops in approximately 2 people per 100,000 every year and has a typical survival period of just 2 - 3 years from diagnosis. It is critical that we discover more about the mechanisms underlying motoneuron loss in MND in order to design novel treatments for this devastating 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 order to development treatments for injury and disease affecting neurons in the spinal cord and brainstem we need a much better understanding of how these systems work in the normal ‘healthy’ state. Our basic research will therefore provide

critical new information that is required to established new ways of treating injuries and diseases which affect movement. We will also undertake research focussed specifically on understanding why neurons die in Motor Neurone Disease (MND). We aim to reveal new targets for the development of novel treatments for this devastating, incurable disease.

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

We expect to use approximately 5000 mice over the 5 years of this project. Most animals will be used or killed as neonatal pups. Due to the use of genetically modified animals, a number of animals need to be bred in order to produce enough offspring with the right genes needed for experiments.

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?

Our experiments will involve the use of nervous system tissue obtained from mice following the killing of animals via humane methods. The severity of the procedures conducted are expected to be subthreshold or mild. Except for mice which act as a model of Motor Neurone Disease, no adverse effects are expected due to the genetic modification of animals. Motor Neurone Disease model mice develop symptoms similar to the human disease including muscle weakness and paralysis. However, we will use animals prior to the onset of significant suffering due to disease symptoms. Animals may be kept up to the point at which they exhibit tremors and alterations in their gait (the way they walk). At the end of experiments all mice will be killed via humane methods.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In order to study and understand the complex nervous systems of mammals it is necessary to study tissue obtained from them. We have recently begun to utilise human stem-cell derived cell culture models to investigate whether basic principles revealed in rodent tissue apply to human cells. However, because cells grown in the lab cannot recreate the complex networks we seek to understand, they cannot completely replace the use of rodent nervous system tissue. There also remain too many unknowns for computer simulations to replace the use of animal tissue.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

One of the key principles of our experimental design is that the maximum amount of data should be obtained from each animal killed thus reducing animal numbers. This is facilitated through the use of isolated spinal cord and brainstem tissue which last up to 6 hours allowing data to be collected from many spinal and brainstem cells per preparation.

Animal numbers will also be reduced in the present study through the use of state-of-the-art genetic and viral techniques. It has historically been difficult to define specific subtypes of neurons for subsequent study. This has meant that investigators have had to record from a large number of neurons, even though they have been attempting to study a small, specific population of neurons. In the present study we will utilise the most recent viral and molecular genetic techniques to label and manipulate discrete populations of neurons. Since we will be able to identify subtypes of neurons prior to studying them, we will be able to reduce the number of recordings, and hence animals, required to meet our objectives.

Refinement

Explain the choice of animals 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.

Refinement

We have chosen to work with mice because we are able to perform analyses in isolated spinal cord and brainstem tissue obtained from them. By using acute, isolated brainstem and spinal cord preparations we can investigate complex neuronal networks while avoiding the pain and suffering associated with experiments on whole animals. We can also utilise many genetic techniques available in mice to design more powerful studies requiring less animals.

Following the breeding of genetically modified mice and/or injection of substances to label or control the activity of specific cells, animals will be used in acute experiments, providing a clear end-point and minimising suffering.

We have also chosen to utilise a mouse model of Motor Neurone Disease (MND) to investigate the reasons why motor neurons die in the disease. Clear end-points are particularly relevant for these MND model mice which develop a neurodegenerative disease later in life. The disease involves progressive motor dysfunction and paralysis. To minimise suffering, we will use ALS model mice for breeding and experimental purposes prior to the onset of significant suffering due to disease.

PROJECT 132

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

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Word limit; 1000 words

Project Title	Myelination in the CNS and PNS
Key Words	myelin, Neurone, inflammation, Axon
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project focuses on formation, maintenance, function of nervous system myelin under normal and disease condition.

In the nervous system, nerve cells communicate with each other via a network of ‘cables’ or axons. Axons are surrounded by an insulating material called myelin that facilitates the rapid transmission of information along them. The cells that produce myelin are called oligodendrocytes (in the brain and spinal cord) and Schwann cells (in the rest of the body). If myelin fails to develop normally or if it breaks down after it has formed, transmission along axons is slowed or halted; potentially causing neurological disability. Primary, genetically determined, abnormalities in myelin formation and/or maintenance are accompanied by changes in neighbouring cells called astrocytes and microglia, and sometimes by axonal injury. The secondary changes in microglia and astrocytes can potentially change the status of these cells from custodians of CNS health to effectors of CNS injury, thus exacerbating the primary abnormalities.

Although we and others have contributed to the understanding of how genetic abnormalities and inflammation contribute to (i) failure of myelin formation and/or maintenance and (ii) axonal injury, the mechanisms, molecules and cells involved are still not fully understood. For example, in the context of specific myelin disorders, it is not known how axonal injury occurs, whether and how microglia cause or exacerbate axon/myelin injury, how abnormal proteins perturb myelin formation/maintenance.

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

Our work will aid understanding of the molecular mechanism by which myelinating cells are maintained and injured and how they ‘speak’ to axons and maintain the

health of neurones. In the long term it should facilitate the development of therapies for the treatment of neurodegenerative disorders such as multiple sclerosis, the leukodystrophies and motor neuron disease.

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

This project utilises mice with spontaneous genetic abnormalities and modifications to understand mechanisms and molecules involved in myelin and axonal injury. Mostly, mice will be culled for tissue collection for characterisation or to generate primary cell cultures. Cell transplantation will be used to assess the myelinating abilities of genetically abnormal oligodendroglia in a normal CNS environment. Injection into the CNS of tracer compounds (molecules that show the movement of materials within neuron) will be used to understand the process of axonal injury. Systemic administration or injection into the CNS of putative pro- or anti-inflammatory factors or demyelinating factors will be used to determine the consequences for oligodendroglia and axons. Approximately 2500 mice will be used over 5 years. Minimum use will be ensured by maximising sample collection from individual animals and from storing samples for future use.

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 receive injections of substances capable of labelling specific cells. The mice will experience mild discomfort during the injection (mild). Animals will be killed by a humane method up to 6 months after injection. For surgical procedures (administration of very small volumes of liquid agents to the nervous system), mice will be anaesthetised using gaseous anaesthetic and analgesic (pain relief) will be administered at the start of the surgery so that it should take effect by the end of surgery (surgery lasts 5 minutes to 1 hour). Post-surgical analgesia will be provided by injection of drugs or through the addition of drugs to the drinking water, as appropriate to the procedure. Administration of substances to the CNS requires lesioning of muscle and bone, which is likely to cause pain. Infections and neurological disturbances caused by CNS injury are extremely rare, in our experience (mild to moderate). Regular monitoring of animals will accompany surgical procedures. Animals will be killed by a humane method some time after surgery. Some mice with gene defects affecting myelin development tremor and seize intermittently (mild to moderate). Some mice will be treated with agents that will cause them to develop signs and pathologies similar to those found in patients with multiple sclerosis. This procedure is rated as severe. Animals that develop long-term symptoms without recovery or symptoms more severe than hind limb paralysis will be killed using a humane method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We will use cell cultures whenever possible, but these do not mimic the situation in man and animals where the nervous system communicates with the immune system and where cells form a highly organised and interconnected 3D structure.

We will review and incorporate alternatives throughout the project as they become available or if considered appropriate.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Experiments will be designed in such a way that we can obtain the maximum amount of information from the minimum number of animals. Advice from statisticians will be sought at the start of new studies. Further, multiple tissues will be collected from individual animals and stored for future use if not required immediately, and shared with other research groups.

Efficient breeding practices (e.g. breeding heterozygous/hemizygous mice to generate control and knockout littermates) will be use to decrease variability between comparators and reduce numbers Genotyping will be carried out early so only suitable animals will be maintained and bred; proven stud males will be reused.

Refinement

Explain the choice of animals 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.

Refinement

We will use transgenic and mutant mice that mimic human disease. We will also use mice with cells and organelles that are labelled with fluorescent markers to allow us to visualise and monitor these cells and organelles in the nervous system. Only mice are currently available with these genetic modifications in the context of a mammalian nervous system.

Mice will be housed in an enriched environment (e.g. with nesting materials and burrowing treats), generally in small groups. Animals undergoing painful procedures will routinely receive anaesthetic.

Observations and objective measurements will be used to identify endpoints and if the endpoint is reached the animals will be killed humanely.

I have over REDACTED experience in the protocols described in the licence, helping ensure efficient use of animals.

Immune-compromised mice will be maintained in barrier conditions to minimise the risk of infection.

Environmental enrichment will be provided routinely.

The NACWO and NVS will be consulted regularly and kept abreast or our work.

We have access to fully equipped small animal theatres for small animal surgery.

PROJECT 133

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Control of strangles in horses
Key Words	Streptococcus equi, horse, virulence, vaccine, 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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project will improve our understanding of how *Streptococcus equi* (*S. equi*) causes strangles in horses and use this knowledge to develop new vaccines and therapies with which to prevent and treat this important 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)?

Strangles is one of the most frequently identified infectious diseases of horses in the world with over 600 outbreaks occurring in the UK each year with many thousands of horses affected. Strangles kills around 2 % of affected animals and is of great welfare and economic concern to the global horse industry. This project will improve the understanding of this disease and test the safety and effectiveness of new vaccines and therapies, which will directly improve the health and welfare of horses in the UK and abroad.

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

Up to 230 horses will be used in this project over a five-year period. This number is significantly lower than used previously, reflecting improvements in our methods, the use of historical control data and real progress towards launching new vaccines for the prevention of this disease. A maximum of 10 horses will be used to provide blood and nasal fluid samples for carrying out laboratory tests to select only those genes or vaccines that are most worthy of further investigation. This will enable us to reduce the number of horses required in subsequent experiments. Up to 60 horses will be used to identify mechanisms important to the way in which S. equi causes disease and to test new therapies that could be used to treat strangles. Up to 60 horses will

be used to test the safety of new vaccines and establish which dose of vaccine is likely to be the most effective prior to challenging horses with S. equi. A maximum of 100 horses will be used to test the effectiveness of new vaccines in studies that will lead to the registration and launch of new products to prevent strangles in horses.

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

The taking of blood or nasal fluid samples will lead to transient discomfort and no lasting harm. These are routine procedures utilised by veterinarians and we have not experienced any adverse effects in taking blood samples or nasal fluid samples over the past 14 years of research. Horses will be maintained on site and may be rehomed at the end of the procedure. Over the course of the five-year period of this project we anticipate that 48 of the 60 horses that are exposed to S. equi will develop early clinical signs of strangles depending on the virulence of the strain used or the effectiveness of new therapies. Clinical signs include swelling of the lymph nodes, an elevated body temperature and a preference for haylage and water over dry pelleted food. These signs are much less serious than those typically experienced by horses that are naturally infected with S. equi and enable us to accurately measure the ability of S. equi to cause disease, whilst minimising the suffering of the animals involved. All horses used in this part of the project will be humanely killed if they fall ill or on reaching the end of the project to ensure that the effects of S. equi infection are minimised and that the infection cannot spread to other animals beyond the group being studied. Where horses need to be humanely killed, they will be sedated, led through to a quiet area away from the other horses and humanely killed through the administration of an overdose of anaesthetic. Approximately 11 of the 60 horses used to test the safety of new vaccines are expected to develop injection site reactions that would normally require treatment by a vet, but in order to minimise suffering, all such horses will be humanely killed as quickly as possible as described above. The remaining 49 horses are expected to have minor reactions to vaccination similar to those normally observed following the administration of a commercial vaccine (slight swelling and pain at the injection site that resolves in a couple of days without treatment). Approximately 25 of the horses given live attenuated vaccines are expected to reach the end of the studies. These horses cannot be rehomed, but could be maintained on site. The remaining 24 horses given protein or killed vaccines may be suitable for rehoming at the end of the study. Approximately half of the 100 horses used to test the effectiveness of new vaccines are expected to develop early clinical signs of strangles as described above. All horses used in this part of the project will be humanely killed if they begin to fall ill, as described above, or on reaching the end of the project to ensure that the effects of S. equi infection are minimised and that the infection cannot spread to other animals beyond the group being studied.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We utilise a wide array of laboratory experiments that are able to answer a number of important questions about the way S. equi behaves without the need to infect horses. These studies enable us to select only those genes or vaccines that are most worthy of further investigation in horses.

However, the use of horses for our research is essential as S. equi only causes strangles in horses and we need to measure how the horse's immune response reacts to the bacteria, therapies and vaccines in order to maximise the level of protection conferred and the benefits of this work. The testing of veterinary products in the target species is a specific regulatory requirement for the approval of veterinary products, which would have the potential to greatly reduce the frequency and number of strangles outbreaks all around the world.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Laboratory experiments enable us to select only the most appropriate strains or vaccines for further testing in the horse, reducing the number of horses required.

The design of all studies will be checked by a statistician to ensure that the smallest numbers of horses are used in order to achieve statistically significant results.

Pilot studies to test the safety of new vaccines will only use three horses in order to minimise the number of horses used whilst guiding the development of new vaccines and optimising the dose to be used.

Infection studies will typically use test groups of between 6 and 9 horses. By optimising the methods we use, we have been able to maximise the reproducibility of early clinical signs of strangles and so will only use control groups of 4 horses. The data obtained will be supplemented with archived data from historical control horses in order to reduce the number of horses required whilst maximising the significance of our findings. We are pioneering the use of historical control data for the reduction of animal use and are currently seeking to revise EU legislation for the regulatory approval of new vaccines, which could reduce the wider use of animals beyond our field of research.

Refinement

Explain the choice of animals 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.

Refinement

S. equi only naturally causes disease in horses. The challenge of mice with S. equi leads to an aggressive septicaemia and pneumonia, which is often fatal and the results obtained from such studies do not necessarily reflect outcome in horses. Therefore, the horse is the only animal that can be used to accurately study S. equi and to measure the effectiveness of new treatments and vaccines. As experimentally infected horses suffer less severe clinical signs of disease than mice, we are able to maximise the value of the data we obtain, whilst minimising the suffering of the animals involved.

The infection system to be used has been refined such that horses develop a gradual onset of clinical signs, which can be carefully monitored up to the point where they reach one of the humane endpoints (lymph nodes swollen or a preference for haylage and water over dry pelleted food). These endpoints are much milder than those experienced by horses suffering natural strangles and enable us to further minimise the suffering of experimentally infected horses whilst ensuring that valuable and relevant data are obtained.

Normally horses will be kept at grass to ensure that they live as they would were they not being used in experimental studies. Horses will be isolated from non-study animals following challenge with S. equi to prevent the spread of infection. The isolation environment in which the horses are kept has been refined to help them maintain as normal a diet and behaviour as is possible within their original peer groups. Our processes are constantly reviewed and improved to maximise the wellbeing of horses whilst they are in our care.

PROJECT 134

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanisms and Therapies in Musculoskeletal Cancer
Key Words	Cancer, Therapy, Musculoskeletal, Bone, Metastasis
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Bone cancer is a potentially devastating condition affecting young adults and companion animals. The most common primary bone cancers – cancers that originate in bone - are osteosarcoma and Ewing's sarcoma. As these tumours grow, they destroy the bone, cause significant pain, and increase the risk of the bone subsequently breaking. If left untreated, the cancer will often spread (metastasise), usually to the lungs, and kill the patient. Current therapies for these cancers are reasonably good but survival rates have now levelled off at around 60% and there is a real need for better and safer therapies. In the work described under this project license, we will start by validating two published and clinically relevant mouse models for studying OSA metastasis, as a foundation for future studies on the molecular drivers that control OSA metastasis.

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 first phase will provide data that clarifies which model is most clinically relevant and where refinements can be applied. When this first phase has been completed, the results will serve as a foundation for additional experiments that will be used to develop safer and more selective therapies for treating and ideally preventing the progression of these cancers. The data collected will be used to guide clinical care in canine and human patients with osteosarcoma. In the longer term the aim is to develop/identify new approaches that will lead to measurable improvements in both quality and quantify of life for bone cancer patients.

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

Model validation studies: 100 mice. Project timeframe is 5 years.

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

The most significant effects on animals from this work relate to the formation of tumours under the skin or in the bone, and subsequent spread to the lungs. In the latter, potential adverse effects relate to risk of fracture, pain and lameness. In both models there could be signs of difficulty breathing (due to lung metastasis) and weight loss/anorexia. We mitigate most of these risks through the use of clear, measurable humane end points. One of our protocols involves surgical removal (amputation) of the hind limb in order to reduce the risk of bone fracture – this protocol is classified as severe, but the effects on the animal will be mitigated through the use of effective pain relieving drugs. Additionally, we will be actively exploring (under this license) a less invasive model that does not require amputation.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We make use of non-animal alternatives wherever possible, including cell culture models for studying the effects of therapy on isolated cells, and computational models for predicting the likely effects of cancer (and cancer therapies) on bone strength and risk of fracture.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

For each cell line, pilot studies will be performed to establish the minimum sample size required for robust statistical analysis. Study design will be optimised to allow sharing of controls across multiple experimental groups. Within each experiment, we maximise data collection from individual animals by using non-invasive imaging and blood tests that can be used repeatedly in the same animal, without the need to kill small numbers of animals at multiple time points.

Refinement

Explain the choice of animals 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.

Refinement

Immune deficient mice provide a reproducible genetic and immune background against which we can study the effects of cancer cells and therapies without the animal's immune system rejecting the foreign cancer cells. The adverse welfare cost to the mice will be minimised by (1) using established anaesthesia and analgesia protocols and (2) monitoring the animals using validated measures of lameness and bone destruction, and (3) using sensitive, validated imaging techniques to identify metastasis, allowing us to remove the animals from the study before they become severely clinically affected. We will also be working to validate a new model for bone cancer that does not involve injection into a long bone and that does not appear to cause pain or lameness.

PROJECT 135

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Fish developmental biology and disease
Key Words	Zebrafish, Medaka, Development, Disease model, in vivo assay
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We aim to address mechanisms controlling development of cell-types, tissues and organs in the body, including the roles and control of stem cells in these processes. Where appropriate we will develop assays to directly address a clinical need related to these studies, for example, correlating patient genotype with the disease symptoms they display, or in vivo drug screening assays to inform our understanding of disease mechanisms and, eventually, to contribute to the development of treatments. To these ends, we will generate and maintain appropriate GA lines, and then use them to investigate underlying cellular and molecular mechanisms of these developmental processes, and then develop appropriate assays. In addition, we will assess how developmental mechanisms influence behavioural patterns, specifically by influencing the ratios of male:females in a breeding population.

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)?

As a result of the work performed here, we will have substantially improved our understanding of basic developmental and disease mechanisms, including cancer and genetic disease, will have developed one or more in vivo assays addressing a clinical concern, and will have increased understanding of the mechanism of regeneration.

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

The number of animals used will be minimised by focusing as much work as possible on embryonic/larval stages prior to free-feeding, but may total up to 111,400 zebrafish and 40,650 medaka over the 5 years. Most will be GA fish, in part because

wherever possible we will use appropriate techniques (e.g. combinations of transgenic lines, recessive mutants) to ensure that only a minimal number of fish are exposed to potentially harmful conditions.

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?

Our choice of fish as our model derives from their being the least sentient genetic model vertebrate organisms. The procedures to be applied are mostly generation and breeding of genetically-altered fish strains, but also isolation of gametes by 'stripping', analysis of labelled cells, introduction of substances into fish, fixation of tissues for microscopic study, study of tumours, and of fin and heart regeneration, and very rarely mutagenesis. Note that almost all protocols are classified as Mild, and the vast majority of these fish will only be exposed to protocols classified as Mild. Wherever possible we will use anaesthetic during stages that may cause suffering to minimise its effects. At the end of the protocols fish will be euthanised by a Schedule 1 method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Development is a process that happens in the highly complex environment of the developing embryo, and also involves interactions with the embryo's environment. As such, this cannot yet be adequately replicated in an in vitro context, although we continue to be open to such possibilities if they are developed. In the context of human development, some tissues/cell-types do not exist in non-vertebrate animals and so cannot be appropriately studied in these models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

As stated, most (90+%) of our work will be on embryos prior to free-feeding animals that are not protected; most of our animal usage will result from generation and maintenance of mutant and GA stocks, which in the vast majority of cases will be harmless in their effects on the adult, and we will use the latest techniques to ensure this is as efficient as possible. Where appropriate, we will use tools such as the NC3R's Experimental Design Assistant to reduce numbers.

Refinement

Explain the choice of animals 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.

Refinement

Fish are the least sentient of the vertebrate models suitable for genetic research. In all our studies we will address effects as early as possible. Anaesthetics will be used wherever appropriate; furthermore, we will refine our use of specific anaesthetics as their advantages and disadvantages are understood. Non-invasive methods will be used wherever possible. Where revised methodology has become available, we will endeavour to update our protocols accordingly. Pilot studies will be used to refine protocols being developed/modified in the lab.

PROJECT 136

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The nature and control of cortical activity
Key Words	epilepsy, neocortex, hippocampus, optogenetics, electrophysiology
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

- We will study how epileptic seizures develop and what effects these have on the brain. Epilepsy is one of the most serious neurological conditions, and many people (estimated at 200,000 in the UK alone) do not respond to currently available treatments.
- There are many things we still do not understand about epilepsy: we do not know how seizures start or how they spontaneously end (which most seizures do, naturally).
- We also have only limited understanding of how seizures affect normal brain function between times, which is highly relevant to why epilepsy is commonly associated with other brain disorders.
- We will apply new technologies for manipulating brain activity, to attempt to modify or even stop seizures.
- The most important of these technologies is optogenetics, which involves getting neurons to make special proteins that can be activated by light. These are normally only found in certain kinds of bacteria and algae, but when they are introduced into neurons, it allows for very precise experimental control over neuronal behaviour. It is possible to activate or inhibit a single cell, or millions simultaneously, just using light.
- Our ultimate aim is to develop this new technology for use in humans, as a brand new way to treat epilepsy.

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

• We anticipate advances in our understanding of the brain’s own natural protective mechanisms, which prevent seizures from starting or spreading, and how seizures usually stop by themselves. • Our studies will also help diagnosis and management

of epilepsy, by indicating new ways to identify and localise the source of seizures in the brain. This is particularly important for severe cases that may be treated surgically. • Our work will also extend our expertise in recording and manipulating brain activity using new technologies such as optogenetics. These new technologies offer powerful and sophisticated new surgical approaches for treating neurological conditions.

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

Up to 4000 mice, and 300 rats over 5 years.

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

• Some animals will have epilepsy (genetic epilepsy, or induced experimentally, by administering certain “epileptogenic” drugs). Once an animals starts to experience seizures, they are defined as being “epileptic” and can then be studied for our research. We do not require that animals are epileptic for long periods for our studies, and all epileptic animals will be used within the first 4 months after developing the condition. • Most animals will only have a small number of seizures that affect a small part of their brain (moderate). These often are manifest only as a subtle pause in the animal’s behaviour, and may be obvious only by recording nerve activity with electrodes in the brain. This is not painful because the brain itself has no pain receptors, and indeed similar recordings are done on human patients. • Other animals will have seizures that spread to the motor parts of the brain and will manifest as motor seizures (behavioural twitches; moderate to severe). • Seizures, by analogy with human reports, are not considered to be painful, but if very frequent, may im-pact on the animal welfare in other ways. We will conduct behavioural tests while recording from the brain, to investigate how seizures affect brain activity both during the seizure itself, and afterwards (“interictal” periods). Animals will be monitored for evidence of marked deterioration in health arising from their epilepsy • The majority of animals will have no neurological phenotype. Some will be carrying genes whose function is ordinarily latent, but which allow the nervous system to be manipulated (e.g. optogenetic genes). These are rated as “mild”. • Some animals will have genes introduced into the brain, by injecting DNA, packaged either in viral vectors, or as plasmids. Our investigations involve recording or imaging brain activity, and using newly developed tools to manipulate brain activity. These recordings will be one of the following: periods of awake behaving recording using wire-less EEG or tethered systems (moderate); imaging experiments of awake, but head-restrained animals (moderate); terminal recordings under anaesthesia; humane killing followed by brain slice recordings (in vitro studies). • We will record the brain activity of some animals under terminal anaesthesia (“mild”). • Some animals will be trained to tolerate head restraint, to facilitate imaging / electrophysiological recordings (“moderate” severity) while they are awake. This will

help us understand the nature of naturally occurring seizures, in an intact, non-sedated brain, the way that seizures occur in humans. • Other animals will be killed humanely for preparation of brain slices for electrophysiology or anatomical studies ("mild" severity).

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Epileptic seizures arise from the combined activity of large populations of neurons and the pattern of activity depends on how these neurons are connected. It is critical therefore to study this activity in its natural environment, in an intact brain.

This information can be supplemented by studies of neuronal cultures, computer simulations and of human recordings, and we will use all these other methodologies wherever possible.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Many of our studies will examine brain activity in the whole animal, recorded either continuously over an extended period of time (EEG), or in repeated recording sessions after training an animal to sit under a special microscope. We will further optimise animal use by preparing post mortem brain slices, relating our findings back to the whole animal recordings in each individual case.

Data obtained from these experiments will be used to develop computer models of seizures, which will help to design future experiments, and simulate treatment regimes.

Refinement

Explain the choice of animals 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.

Refinement

- We will primarily use mice, because they allow simple genetic manipulations, either to create genetic models of epilepsy, or to introduce genes which allow experimental control of neurons (eg. optogenetics).
- Genetic models are a highly refined way of reproducing human conditions, in instances where a particular mutation has been shown to be associated with epilepsy.
- Other studies will involve experimentally induced epilepsy, using two models which have been widely used in the epilepsy research community, and are considered to be refined models of epilepsy in that they reliably result in regular seizures, without excessive mortality or reduced life quality.
- Finally, in other studies, we will study seizure-like activity induced in brain slices. In these experiments, suffering is minimised because epileptic activity is induced only after the animal has been humanely killed.

PROJECT 137

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Oxidative stress and inflammation in retinal degeneration
Key Words	Mouse, Retinal degeneration, Oxidative stress, Inflammation
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Retina is at back of our eyes and contains light-sensitive cells (photoreceptors). Retinal degeneration is characterized by the death of photoreceptors. In this project we aim to understand the pathogenesis of retinal degeneration and to develop therapeutic strategies for patients with retinal degeneration.

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)?

Results from our project will benefit scientific communities, clinicians, patients and their family members.

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

We plan use mouse for our project and the approximate number is 1600 for 5 years.

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

We will induce retinal degeneration (e.g. diabetic retinopathy) in mice or use genetic modified retinal degeneration mice for elucidating the disease mechanisms and developing new treatment for retinal degeneration. The level of severity is expected to be mild or moderate. After the experiments, mice will be killed by Schedule one method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We have used *in vitro* mammalian cell systems to characterise the functional role of oxidative damage and inflammation and evaluate the protective role of potential drug candidates. However *in vitro* assays cannot adequately characterise the effects of oxidative damage and consequent inflammation. It is not feasible produce an adequate model for a functional retina in vitro, and therefore we need to use living models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have consulted the statistician about minimum number to achieve significant difference. Power calculations, based on our combined prior experiences with murine models, reveal that eight animals per group will yield sufficient data to achieve biologically and statistically significant results.

Refinement

Explain the choice of animals 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.

Refinement

Mouse has been commonly used to study the pathogenesis of retinal diseases, the phenotypes of mouse retinal diseases reflect the pathologies seen in man.

Animal under study will be housed in family grouping with regular health checks by our NVS performed in lines with the general running practice of the unit. At the end of each experiment animals will be humanely killed by a high dose of anaesthetic (Schedule 1 method).

PROJECT 138

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Research into infectious fish diseases
Key Words	fish disease
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To better understand, diagnose, control and prevent fish diseases, thus improving production and welfare of farmed fish and protecting wild aquatic life.

There are two main sub-objectives:

a) To improve understanding of aquatic animal disease (host susceptibility, infectivity, and pathogenicity);

b) To develop and apply methods examining the efficacy of substances for therapeutic and/or diagnostic use

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)?

With the ongoing depletion of wild fish stocks, fish farming is increasingly a critical sector for aquatic food security. Despite major advances over the last 40 years, infectious diseases continue to be a major constraint, reducing productivity, fish welfare and resource use efficiency. Fish farms are usually in contact with the surrounding river/sea environment, meaning infections can move easily between farmed and wild stocks. Research to understand diseases and develop control methods (e.g. prophylactic treatments, vaccines, disease resistant strains) is integral to assuring the future sustainability of aquaculture. The UK has a high aquatic health status, which is under constant threat from emerging and introduced diseases. Government policy is to eradicate any notifiable diseases disease by slaughter and disinfection if possible. This policy requires reliable validated diagnostic methods and an understanding of disease risks to wild fish populations. Maintenance of the UK's aquatic biosecurity and compliance with national and EU legislation on aquatic disease requires knowledge of aquatic disease supported by long-term programmes of diagnostic tool development, disease monitoring, disease control and prophylaxis.

These form the statutory and scientific basis of the aquatic animal disease research programmes for wild and farmed fish covered by this licence.

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

We seek authority to work with any fish species because fish from different environments and continents contribute to food security. In terms of wild fish disease research, we are likely to use endemic species. We seek authority to use a maximum number of 99,000 fish over a 5-year period; however, this number is expected to be much lower as it includes a large contingency in case a fish disease outbreak occurs requiring additional investigations.

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?

One main procedure is used in four of the six protocols in this licence; this involves pathogen challenge, i.e. controlled exposure to pathogens. By nature of the serious pathogens of interest, the potential adverse effects are generally severe. The actual adverse effects are managed by defined humane end-points implemented by intensive monitoring which involves both direct visual checks and REDACTED

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The development of disease or resistance is difficult to study without a whole animal model as it involves multiple tissues and organs. The early parts of our investigations are conducted in non-animal models; however, the infection, pathogenesis, host immune response, treatment and vaccination responses require complex metabolic, anatomical and immunological mechanisms that cannot yet be modelled in vitro or in surrogate invertebrate species.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Every effort is made to minimise the numbers of animals used in studies: statisticians advise on the numbers required to achieve meaningful results, animal husbandry experts advise on fish social needs and the ethics committee (AWERB) scrutinises each study plan. Members of the AWERB include scientists, veterinary surgeons,

animal husbandry experts and lay people; they all have the power to veto study plans.

Refinement

Explain the choice of animals 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.

Refinement

We want to have the ability to work with any fish species, of interest to both conservation and food production. The main tool we will employ to refine prospective severe procedures and minimise suffering is **close monitoring**.

Fish are typically sourced from our own breeding establishment to ensure disease-free, high quality animals acclimated to experimental tank conditions. Externally sourced fish are health screened on arrival and quarantined to ensure a good health status and acclimation before use. We have a dedicated, high-tech aquarium facility, with monitoring and call-out alarms (water temperature, flow, depth). Named persons oversee staff training and performance, care of fish, and dissemination of information. Close links with the international fish research community ensures we are aware of any developments in fish care and biosecurity. Consideration is given to all aspects of the environment (including enrichment) e.g. space, water quality and current, conspecific density, lighting, shading, refuges and diet. We have a dedicated team of specialist aquarists complemented by long-standing experience in fish husbandry. Stock and experimental fish are closely monitored and interventions (including veterinary treatments) are implemented wherever possible. We believe we have a strong institutional culture of care and have review processes to identify where improvements in care can be made.

PROJECT 139

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Breeding livestock for traits derived from new technologies
Key Words	Computer tomography, genetic, genomic, sheep, pigs
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aim of the research is to develop the mechanisms to allow livestock species to be bred for new traits with the use of innovative technologies, making breeding programmes more effective and sustainable. The scientific outputs will be measurements or accurate predictions of growth, body composition, meat quality, health and welfare traits, which can be used alongside pedigree information to make informed selection decisions of which animals to use for breeding the next generation.

The objectives will be to:

- use advanced, non-invasive, body composition analysis techniques of live sheep and pigs to develop, validate and standardise suitable novel measurement techniques for important traits that are difficult to measure. These traits include body composition, meat and carcass quality predictors, body dimensions or volumes that relate to lambing ease or greenhouse gas emissions.
- develop and validate markers found in blood or other DNA sources to predict resistance to worms and other disease (e.g. lameness, mastitis), or that can be used as welfare indicators in sheep.
- apply these methods on sufficiently powerful numbers of animals with pedigree information to understand their genetic control and potentially develop DNA tests for these traits
- use these methods to incorporate important product quality and welfare-related traits into breeding programmes for sustainable breeding of livestock species.

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 long term it is expected that the programme of work will deliver higher production efficiency and welfare of sheep and pigs. In particular, it will provide in the short-medium term: • novel knowledge on the genetic basis of new traits that could be included in livestock breeding programmes • harmonised and robust methods to measure these traits in across large numbers of animals • capability to employ genetic or genomic (DNA-based) selection for traits that have previously proven difficult or expensive to measure, including meat and carcass quality, spine characteristics, disease/parasite resistance, welfare indicators.

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

The work will be conducted on sheep and pigs. It is anticipated that around 6000 sheep and up to 200 pigs will be CT scanned and a further 6000 sheep will have DNA and/or faecal samples taken over the 5 year duration of the project.

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?

Central to the achievement of the project objectives are three experimental procedures: advanced, non-invasive imaging of live sheep and pigs for genetic or genomic selection, or to understand interactions with management regime; DNA sampling (e.g. nasal or saliva swabbing, blood sampling, or ear punch) for DNA extraction or to look for biomarkers; and individual faecal sampling. Most animals will only undergo one or two of these procedures once in their lifetime, although some may get multiple procedures over time to look for physiological changes. Otherwise animals will be managed as in commercial sheep flocks or pig herds. No adverse effects are expected from these procedures and it is anticipated that animals will be returned to their home flock/herd, and will be fit to be slaughtered or sold for breeding, if appropriate.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Since we need to measure these traits in the target species to enable genetic selection, and look for biological and genetic markers in the relevant species, the work needs to be done on animals and the proposed goals cannot be achieved by using any alternatives. Wherever possible we use our extensive databases containing historical data of archived material from previous body composition

analysis procedures and of meat joints, but as we are now extending studies into other breeds and lines, there are limited opportunities for replacement for most elements of the work.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Consultation with statisticians ensures that the minimum number of animals are used to enable robust scientific conclusions to be drawn. Where possible, on both ethical and economic grounds, our research is based on re-analysis of existing data, or data sharing with other institutes. We maintain well-organised, comprehensive computer-based databases for the type of experiments described here. This means that: (i) historical data is readily available for re-analysis, where this is relevant (thus reducing or replacing the need for animals in some cases), and (ii) we have good procedures for checking whether animals which have been subjected to particular experimental protocols have performed in a different way from others (allowing refinement of procedures if necessary).

Refinement

a of animals minimises stress to the animals. Use of mobile imaging equipment reduces the need for excessive animal transport to travel and means that we can take it to farms with appropriate handling systems. The equipment has recently been replaced with an higher specification instrument which gathers data more quickly, reducing the acquisition time, so that the procedure for each animal is faster and more reliable.

We are constantly looking for minimally invasive ways of collecting samples or data. For example, the use of saliva / or nasal swabbing to replace faecal and blood sampling are used where possible.

PROJECT 140

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Production and Maintenance of Genetically Altered Mice
Key Words	Production, Genetically Altered, Archiving
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The production of genetically altered (GA) animals to unravel the functional role of genes employs a variety of model organisms amenable to gene manipulation. Incredible advances in technologies over the last 20 years have enabled many subtle and controllable genetic manipulations of the mouse genome. The mouse itself is essential to our research because, as a mammal, it shares many of the developmental milestones and disease states that we, as humans, will experience in our lifetimes. Changes in the genes of the mouse can closely mimic the changes seen in human disease as the mouse has the same basic tissues and organs, and shares much of its physiology with humans. Through this work we will better understand the role of genes in human disease, and allow us to examine gene activity during development in the context of other genes, other cells, other tissues.

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)?

All models that are created are aligned to the goals and aspirations of the other projects this licence will support thus minimising animal usage at this stage of their own projects. The expertise available to us and the application of well-developed strategies ensures high quality mouse models with targeted and, where possible, standardised mutations. We actively share our knowledge and resources with the scientific community to allow them to take advantage of the production processes we have optimised. Through the use of GA mice, the effects of an altered gene can be studied in great detail, and provides us with a window into its biological role and in turn an insight in to its role in disease.

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

Mouse 450,000 animals predominantly for breeding and production of GA mice. The vast majority of these mice (>90%) are expected to remain below the mild severity limit. 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?

This is a service licence for our Institute that will generate GA mice. We alter the DNA of the mice by microinjection of genetically modified stem cells or nucleases such as CRISPR that alter the DNA sequence in a targeted manner. This is done in early stage embryos. These embryos are generated via superovulation by intraperitoneal injection of hormones into donor mice. After microinjection embryos are replaced surgically into a pseudopregnant recipient to develop to term. They are made pseudopregnant by mating to a male that has previously been surgically vasectomised. All surgeries use a general anaesthesia and analgesics followed by post-operative monitoring from trained and skilled technicians. We breed selected mice born from these embryo transfers, including GA mice to generate cohorts for phenotypic analysis to help understand the effect of the genetic alteration or for use on other project licences at the institute for further in depth analysis. The vast majority of mice will show no adverse effects with less than 10% showing some harmful effects caused by the genetic alteration. The harmful effects caused will be addressed where possible with husbandry and veterinary support. Where this will not help, the mice will be humanely killed. Sperm from each novel genetically altered line will be cryopreserved and sent to international repositories to be shared with other researchers around the world for more detailed study. An in vitro test of each frozen line is carried out to ensure that it is still capable of fertilisation post freezing (IVF). During the course of the project, if at any stage an animal experiences adverse effects that cannot be ameliorated, it will be killed humanely and in a timely manner. All animals that have reached the end of their study will be killed using a humane method of killing.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Extensive *ex vivo* analysis is integral to all at our Establishment and is always the first option considered when new biological areas of interest are identified. However to study the full effect of a gene mutation it is essential to study it in context with all molecular, developmental and physiological interactions provided within a living

mammalian system. Due to its similarity and availability of extensive genetic manipulation techniques, the mouse is ideally suited to allow us to study these interactions.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We continuously look at ways to minimise the number of animals used to propagate mutant models. Embryo numbers and recipients are carefully aligned to the number of clones required, while efficiency rates are routinely monitored ensuring the fewest number of animals are required to obtain the required number of new mutant models. Archiving has moved to using sperm as the predominant method of cryopreservation, again reducing the number of animals required to secure a line. These cryopreserved lines are deposited for distribution to the scientific community allowing for a global offset of our production rates by reducing the need to reproduce a mutation at other REDACTED . We have calculated that each colony we produce requires 300 mice to reach a stable colony. By distributing and archiving in sustainable archive we have been able to potentially reduce the global production by around 750,000 animals should all colonies be recovered and progressed. The development of new gene editing technology (CRISPR/Cas9) should bring benefit both to the reduction of the numbers of animals used and refinement in the ability to create more bespoke mutations

Refinement

Explain the choice of animals 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.

Refinement

Comparative anatomical, embryological and physiological studies have shown that mice and humans have the same basic organ systems, skeleton and reproductive cycles. These similarities, coupled with the rapid advances in technologies available to manipulate the mouse genome, make the mouse the most suitable model to mimic human disease condition.

Mice are only created if they are required for experimental analysis in line with the programs of the establishment. Careful monitoring and adaptation of our processes has led to refinement of the stages of the processes required for the maintenance and provision for experimental purposes. This has seen us reduce the average cage holding within the facility from 19 to 13 per colony showing elements of reduction driven by refinement. This has been underpinned by the development of software

that allows us to have greater oversight on the operation and requirements of large scale production. This is now being made available to other establishments to allow them to also gain such benefits.

On-going review of breeding and production data coupled with standardised welfare observations, allow us to further refine procedural, production and breeding protocols both within this licence and in the provision of optimal breeding strategies for other project licences at the establishment.

Husbandry and health monitoring of all animals under this licence is performed by a team of highly competent Animal and Scientific Technicians that are assessed under the Institutes competency assessment program. Cleaning regimes are minimised to ensure stress and disturbance to breeding and stock animals is reduced. Environmental enrichment is provided to account for the individual needs of the animals e.g. nestlets for nest making by pregnant or lactating females.. All animals will be group housed where possible.

All surgical techniques will look to adopt the principles of aseptic techniques as described in the LASA Guiding Principles for Preparing and Undertaking Aseptic Surgery
http://www.lasa.co.uk/pdf/lasa_guiding_principles_aseptic_surgery_2010.2.pdf

PROJECT 141

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Role of blood vessels in regulating bone physiology
Key Words	Blood vessels,, Bone,, Ageing,, Haematopoiesis,, Microenvironment
Expected duration of the project	5 year(s) 0 months

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

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

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Skeletal ageing characterized by decreased formation of bone and impaired formation of blood cellular components is associated with changes in the bone vasculature. However, the physiological factors regulating these age dependent changes in the bone vasculature are unknown. We use both computational and experimental approaches to identify novel mechanisms involved in skeletal ageing. I therefore propose to elucidate aetiology and consequences of ageing in the bone marrow microenvironment using the mouse as a model system with the following aims:

Aim 1. To investigate the role of physiological factors relating to age dependent bone loss.

Aim 2. To understand the regenerative and therapeutic potential of blood vessels in bone: Bone vasculature plays a critical role in fracture repair, wound healing and irradiation injury. Therefore it is important to understand the regenerative potential of blood vessels to regulate age related bone loss.

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 proposed research has immense potential in clinic and industry by uncovering novel targets for diagnostic and treatment of age related skeletal and vascular conditions. The investigation of molecular and cellular components which regulate stability of systems within bone marrow contributes to our understanding of the origin and consequences of age related physiological changes in the skeletal system. We are also collaborating with translational laboratories to evaluate the potential of specialised capillaries in clinical settings.

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

Mice: 3220/year for 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?

Our protocols will be of a mild to moderate level of severity. In brief we will be inducing pathologies with administration of pharmacological drugs and/or chemical/genetic manipulation. We will then try to reverse disease using drug treatment, and/or chemical/genetic manipulation. Treatment and monitoring of pathologies will require the use of procedures similar to those used in human patients. Many of the experiments are ex vivo, in which case the animal is killed to obtain tissue. In the case of in vivo experiments, animals are killed at the end of the experiment, typically followed by further experimental analysis (i.e. anatomical or molecular). Animals will be killed by a humane method at the end of the project period. Animals exhibiting any unexpected harmful will be killed, or in the case of individual animals of particular scientific interest, advice will be sought from NACWO, NVS or the local Home Office Inspector. If the animal fails to respond to treatment or its condition deteriorates, it will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Skeletal ageing is a physiological condition that alters a number of factors in bone and its microenvironments. The underlying reasons for this and the influences on the bone marrow microenvironments are not very well understood. These interactions are impossible to accurately replicate *in vitro* at the present time with tissue culture models, and there are no in vitro models available that can model the effects of ageing.

Genetically altered mice are currently the only system available for analysing how mammalian genes work in the complex environment of the living organism. Where possible, we make use of in vitro cultured cell lines to carry out pilot experiments to gain some information about the behaviour of genes we have constructed before hypotheses are tested in mice. In addition, we use ex vivo culture systems of cells isolated from genetically modified mice to replace procedures that would otherwise be carried out in mice, where this is feasible.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

At every stage in our experiments, consideration will be given to ways in which we can reduce the number of animals. Several of the protocols that we use are designed in such a way to obtain the maximum possible data from a single animal.

Genetically modified animals will be generated by the highly experienced staff of our dedicated transgenic facility. Best practice will be followed but it is difficult to predict how many animals will be required as this depends on the how the genes that we have constructed behave. Where genetically modified animals are generated in house, established breeding practices will be followed that are designed to generate the minimum number of animals needed.

We will consult with statistical experts within the institute when planning to ensure experimental group sizes are no larger than they need to be to obtain robust experimental data. For most of the quantitative experiments, sample sizes will be set using statistical analysis, otherwise, we will use the least number of animals to provide an adequate description, generally on the basis of previous experience. In terms of the numbers of animals required, usually 6-10 animals per treatment group are sufficient to obtain the required results. In addition, we will use the NC3Rs ARRIVE guidelines in reporting our results, and in considering the design of our experiments (e.g. allocation of animals to groups, blinding, etc).

Refinement

Explain the choice of animals 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.

Refinement

The similarity of physiological mechanisms between mice and humans makes them an excellent system for gaining insights into the regulation of ageing in humans. Moreover, they share similar endocrine systems to humans and therefore allow for extrapolation to human disorders. All the procedures in this licence are classified as either mild or moderate and are done under local, general or terminal anaesthesia, where appropriate, to minimise stress and suffering of the animals. In addition, when appropriate, analgesia will be provided.

To administer pharmacological treatments, and especially when medium-long term treatment is needed, multiple injections will be replaced by osmotic minipumps which are less invasive, whenever possible.

Animals will be kept warm and monitored regularly during and after anaesthesia. Peri- and post-operative analgesia will be provided; agents will be administered as

agreed in advance with the named veterinary surgeon. Any animals exhibiting signs of pain, distress or significant ill health will be humanely killed. We will regularly consult with animal care staff, vets and colleagues about best practice and potential further refinement of our procedures.

PROJECT 142

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Biology and treatment of CNS neoplasia
Key Words	Brain tumour, Neural development, Stem cells, Transgenic mouse, Glioblastoma
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aim of the project is to better understand where brain tumours come from and how they grow within the brain. It is already known, that a small population of cells in the brain (so-called stem cells) can give rise to brain tumours. When growth promoting genes are getting out of control in these stem cells, they can cause these cells to form tumours.

The specific aims are to use the same genes and their mutations that are found in human tumours and insert them into these stem cells.

When a patient presents with a brain tumour, these brain tumours would already be in an advanced stage, and therefore not only carry a single mutation (which may be responsible for its initial growth) but also many other mutations.

We therefore want to use a mouse model where we can introduce this mutation under highly controlled conditions and within a well-defined environment. Only under such conditions it is possible to study the next steps, i.e. how a tumour develops and progresses from a stem cell.

It is important to understand these next steps, to be able to design a drug or reagent for the treatment.

This can be tested by applying a novel treatment alone, or in combination with known modes of treatment such as radiation or chemotherapy.

We are also interested in studying the brain development, because the genes and processes involved in the development of the normal brain also play a role in brain tumours. It is known, that basically a cancer cell hijacks the processes that are

normally used to develop a immature brain cells into a functioning brain neuron. By doing so, the normal process (often also known as pathway) loses its normal function and is now involved in tumour formation. These changes and a pathway are often caused by mutations in the proteins that are important for the proper functioning of these pathways.

Understanding how a mechanism important in brain development is regulated will also help us understanding how a normal brain stem cell can become a cancer 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)?

This project furthers our understanding of how brain tumours progress from low to high grades, and how functions that are important for the growth and development of the normal brain are related to the formation of a brain tumour from a normal brain stem cell. Once we understand these processes better, we can use known or newly developed treatment to halt these processes in order to stop brain tumours growing.

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

We intend to use genetically modified mice including immunosuppressed mice for cancer stem cell grafts, and also mice with genetically modified “tumour suppressor” genes and “oncogenes”. We intend to use 10,000 animals over the next 5 years.

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

The main aim of this project is to understand the growth of brain tumours in a mouse model. Therefore, our experiments need to form cancer cells in the brain, which eventually will grow to tumours. Brain tumour has a side effect on the behaviour of these animals. These include weight loss, reduced movements, and withdraw from the group. Therefore, our trained animal staff monitor these animals on a regular basis (twice daily) and specifically watch out for these signs. Once a mouse shows such a sign, it will be humanely killed and used for the experiments, which includes a thorough tumour analysis with molecular biology methods, growing cells from it or the analysis by a pathologist. The expected level of severity in the above circumstances is moderate.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Complex diseases such as brain development and tumours can only be studied in whole organisms. We use cell culture where possible, but to study tumour origin from a stem cell within the living brain and to follow how cancer cells migrate through the brain, an animal model must be used.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

1) By using live animal imaging (for example MRI, magnetic resonance imaging), we can follow the growth of tumours at various time points in development without the need of killing these mice, thus reducing the number of mice.

2) We derive stem cells from genetically modified mice, which can then be further studied in cell culture.

3) We have determined that 12 mice are needed per group in our xenograft studies (transplantation of human cells into mice) in order to achieve statistically significant and reproducible results.

4) We constantly evaluate our methodologies and aim at best practice in relation to the 3Rs. For example, we found that by injecting a new type of virus into newborn mice significantly speed up the development of the tumour. Previously it took nearly one year to form a tumour, and now it is just above a month. Also, the rate of tumour formation is now nearly 90%, in comparison with 20% previously. This considerably saves on mouse numbers and on cost.

5) we have developed a method where we transfer genes into the brain of embryos with a very mild electric shock. This replaces complex breeding schemes and saves on mouse numbers and on cost

Refinement

Explain the choice of animals 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.

Refinement

The mouse is a well characterized model system to mimic human diseases. We have chosen to use a genetic approach to study brain tumour formation, as it allows for controlled and predictable experimental conditions as compared for example to carcinogen-induced mutagenesis, which has been often used in the past.

The twice daily surveillance scheme in our animal facility and specifically trained staff ensure neurological signs of brain tumour development are discovered in an early clinical stage.

We constantly refine and re-evaluate our protocols to minimise harm to the animals. For example, we have established a method of injecting a tamoxifen metabolite directly into the brain to specifically target the cells of interest, therefore reducing side effects in the entire body. We also trialled the use of an osmotic mini-pump to provide continuous and localised administration of drugs to prevent the need for repeated daily injections, and to provide a steady and controlled administration of the drugs in comparison to a concentrated dose.

PROJECT 143

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Skin and hair follicle development and regeneration
Key Words	skin, hair follicle, stem cells, regeneration and wound healing, epithelial-mesenchymal interactions and transdifferentiation
Expected duration of the project	0 year(s) 6 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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overarching aim of our work is to define new biological properties and potential therapeutic capabilities of specific skin and skin appendage cell subpopulations. Thus we aim to:

- Identify cellular and molecular mechanisms involved in the development and maintenance of hair follicles and skin fat
- Define what distinguishes follicle cells in the context of wound healing, regeneration, induction of new structures and cell reprogramming.

Investigate the potential of hair follicle derived cells for skin and hair follicle replacement, and for cell therapy in treating the skin disease recessive dystrophic epidermolysis bullosa.

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 inform us about the development of different cell types in skin and hair follicles, and specifically the labelling of cells permits us to determine what particular populations turn into as the skin grows, and whether stem cells are present. It will also inform us about the what controls epithelial stem cell activity. Ultimate benefits could include refinement of isolation of mesenchymal stem cells from skin adipose tissue, and the ability to create new skin tissues and even related epithelial structures such as the cornea of the eye, for transplantation.

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

We will use mice, up to 400 over a six month period.

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?

As we are breeding mice with a harmless marker gene there are no expected adverse effects, and the expected level of severity is mild. Animals not used will be killed or the colony maintained if a new licence is applied for and obtained.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We do use non-animal culture methods as part of the experimental regime, however there is no alternative to employing gentically labelled cells in order to follow what the cells turn into when subjected to various treatments, and when mixed with non-labelled cells. We also need to use immunocompromised mice to host the long term development of recombined tissues. We rountinely use organ culture methods for short term experiments but need the animal work to investgate long term fates of cells/stem cells.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The in-vitro (culture) work that is done in parallel with this research, reduces the number of mice used in our studies. Breeding of the mice will be tightly monitored and controlled depending on the requirements of th user. By virtue of staff having experience and knowledge of the particular line that is being kept, the usage of the animals can be optimised.

Refinement

Explain the choice of animals 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.

Refinement

Mice have been selected for this work as they are the species of choice for genetic manipulation,cell labelling and ease of manipulation. Mice will be cared for by dedicated staff in the animal unit with all the requisite training and skills needed to breed and maintain the animals. These individuals will also closely monitor the

health of animals that have undergone surgery. In the event that any welfare issues arise they will be addressed at an early stage, and suitable end points will be established by consulting with the NACWO. Only animals incorporating a harmless genetic label will be bred on this licence, at the mild severity level.

PROJECT 144

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The impact of stressors on welfare, fertility and production in cattle
Key Words	Stress, welfare, fertility, reproduction, cattle
Expected duration of the project	5 year(s) 0 months

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

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

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To identify the key factors that affect fertility in dairy cattle, how they work, relative impacts and to determine possible strategies to improve them, including short-term pharmacological treatments, longer-term changes in management practices and genetic improvement for improved fertility and reduced susceptibility to stress

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

Our research underlines the need for improvements in management and welfare in a way directly applicable to the farming industry and understood by farmers as a loss of production as well as poor welfare. Understanding the underlying mechanisms will enable new prevention strategies and treatments to be formulated including long term genetic improvement of dairy cattle. Findings may also shed light on mechanisms relevant to stress and fertility in other species. Sub-fertility due to stress is a recognised problem in human medicine. This work will thus also have potential benefits for human reproduction.

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

Cattle. Up to 6000 may be monitored with 500 of these undergoing more regular blood sampling and examinations.

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

Animals will be treated in the normal way they are on commercials farms. Any disease will be treated in the normal method used on that farm. The additional

sampling such as blood samples or small biopsies that will be of mild or moderate severity and not dissimilar to those used for clinical and diagnostic purposes.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

It is essential to carry out these studies in whole animals due to the interaction between stimuli and the physiological state of the whole animal. The naturally at risk population will be studied and only mild stress of a similar intensity to that experienced under normal management will be artificially induced in any animal.

In vitro studies, usually using animal tissue collected from abattoirs, have and will be used to study some of the underpinning physiology with animal studies concentrating on whole animal and stress interaction.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Studies will employ as few animals as statistically necessary and sample size calculations and analysis of preliminary data as animals are enrolled sequentially will be used to determine if the study will actually yield the expected results.

Refinement

Explain the choice of animals 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.

Refinement

In the cow unlike other species, it is possible to monitor follicular growth trans-rectally. Some data will be collected at the same time as examinations for management purposes. Use of animals already subject to management stressors on commercial farms will reduce the need to artificially expose experimental animals to stressors. This is an opportunistic approach, describing the effect of stress and modelling explanatory variable. Animals will be treated using existing protocols on the farm so animals may be treated sooner due to the studies, but not later. The use of lactating clinical cases will also permit collection of milk samples for daily

monitoring of some hormones thus reducing the need for repeated blood sampling with a needle.

PROJECT 145

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Metabolic sensing and energy homeostasis
Key Words	Obesity, metabolic diseases, brain, neurocircuits
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Obesity is a disease of brain pathways regulating appetite. These pathways rely on sensing mechanisms to detect how much energy is available in the body and what kind of nutrients are available for biological functions. Brain nutrient and energy sensing pathways are poorly characterized, which hinders our ability to develop safe and efficient drugs to prevent and treat obesity. In this project, we want to characterize brain pathways sensing proteins that are important in the regulation of energy balance. We know that dietary proteins promote satiety and leanness, but how the brain detects proteins and how this detection modulates hunger and satiety is poorly understood. Characterizing these pathways will increase our knowledge of brain pathways regulating appetite and metabolism and may lead to the discovery of new research avenues to develop safe and efficient drugs in the treatment of obesity and associated metabolic diseases.

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

Obesity represents a major threat to public health, as it is a major risk factor for premature mortality from cardiovascular diseases and certain cancers. The direct costs of treating overweight and obese people are constantly increasing (£4.2 billion in 2007) while indirect costs reached £27 billion in 2015. There is currently no safe and efficient drug therapy to prevent or treat obesity. The aim of this project is to understand how the brain senses proteins and how this sensing regulates appetite to identify new research avenues for safe and efficient anti-obesity drugs. Direct benefits that will likely arise from this work: increased knowledge and understanding of how the brain senses proteins. These findings will be used by our group and other researchers to further study the biology of brain protein sensing. We will also identify how brain pathways sensing proteins interact with brain pathways sensing energy and other nutrients, to determine if targeting distinct brain pathways in combination

could produce greater health benefits. Last, we will determine how brain protein sensing produce a coordinated regulation of appetite and metabolism, to better understand how the body fights against weight loss during chronic energy restriction. Indirect medium-term benefit: Our findings will lay the foundation for follow-up preclinical and clinical research. They will identify candidate therapies directly targeting brain protein sensing mechanisms, pathways integrating protein and energy sensing, and pathways coupling energy expenditure and appetite. REDACTED studying the genetics of obesity in Humans to see if the pathways and genes we have identified are associated with obesity or metabolic diseases in the human population. In the long-term, our findings may contribute to the development of efficient treatments for human obesity.

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

I expect to use around 6000 wild-type and transgenic mice over 5 years.

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

The majority of animals (90%) are not expected to show signs of adverse effects that impact materially on their general well-being, and may transiently show moderate clinical signs (piloerection, reduced activity). Very rarely the severity of these signs may be such that the humane end points may be reached. Animals are monitored on a regular basis to detect any sign of distress or suffering. Analgesic agents will be administered as required. At the end, all animals will be killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We need to use whole organisms because the control of energy balance and metabolism occurs at the level of the “whole organism” and not simply at a cellular level. We need to use mammals to model how the human brain works because mammals have unique sophisticated pathways to regulate feeding and metabolism. However, we have developed and continue the development and use of primary cell culture, immortalized cell lines and brain explants to model primary sensing mechanisms and intracellular signalling pathways in vitro.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We need to use whole organisms because the control of energy balance and metabolism occurs at the level of the “whole organism” and not simply at a cellular level. We need to use mammals to model how the human brain works because mammals have unique sophisticated pathways to regulate feeding and metabolism. However, we have developed and continue the development and use of primary cell culture, immortalized cell lines and brain explants to model primary sensing mechanisms and intracellular signalling pathways in vitro.

Refinement

Explain the choice of animals 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.

Refinement

Rodents allow the study of whole body control of energy balance in a manner relevant to humans, as pathways involved in the control of appetite and body weight are largely similar between rodents and humans. Rodents allow access to several tissues critical to the control of metabolism (brain, pancreas) that are inaccessible in humans. Rodents are amenable to genetic manipulations, offering endless possibilities to characterize mechanisms underlying diseases in a specific and relevant manner.

We will take a number of measures to refine our use of rodents and minimize welfare costs. Animals will be housed according to the best recommendations in a size appropriate environment with shelters and nesting materials. Tubes to act as hiding tunnels and shredding toys and wooden chewing toys for animals to gnaw on will also be supplied. When not having food intake actively measured, food will also be hidden in bedding and floor covering to give the animals the opportunity to forage. Health and welfare will be assessed daily by competent staffs to detect any upcoming problem at an early stage. By performing pilot studies and choosing well established protocols based on extensive previous experience, we will minimize the unknown effects on the mice and subsequently pain, distress and suffering. We will use non-invasive techniques wherever possible and use pain management when appropriate.

PROJECT 146

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Taste perception in rodents
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Taste is the least studied of all the senses. And yet, it plays a crucial role in the dietary choices that animals, including humans, make. Given worldwide concern about the current dietary choices in human populations and the obesity crisis, it is perhaps surprising that so little is known about the mechanisms of taste perception, and particularly how it is affected by the stressful experiences of an animal.

This project investigates how stress, anxiety and depression might affect how animals respond to different tastes. It then applies that knowledge to see if it is possible to develop novel ways to measure the welfare of laboratory rats and mice based on how they respond to tastants. This could provide a new way to assess their welfare.

The objectives of the project are to:

1. Understand more about how stressors can alter taste perception;
2. Understand if animals' behaviours towards different tastes can be used to measure welfare in laboratory animals.

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)?

Knowing how animals learn to use flavours to select the foods they eat is important in the context of food choices by humans. Modern foods often make use of synthetic flavours, which may make it hard for the body to learn to regulate the intake of nutrients. In addition, stress could influence how we learn about foods. It is important to know more about the role that taste plays in determining what we eat. The project is also highly relevant for animal welfare, as it will describe the effectiveness of two novel techniques to measure welfare using taste perception. This could lead to new ways to measure the welfare of laboratory rodents.

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

Mice – 1800 over 5 years Rats – 1800 over 5 years

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

Each animal will undergo a single manipulation that is predicted to affect its stress levels and welfare (either positively or negatively), or act as a control. Before, during and after a manipulation, animals' responses to different tastants will be measured. Animals may also undergo behavioural tests to assess how stressed, anxious or depressed they may have become to further validate our measure. Individual animals may experience pain or distress from dietary or stress manipulations, or from injections (sometimes repeatedly). Each animal will undergo a single manipulation of stress. The level of severity for those animals will be mild or moderate. The animals will be humanely killed at the end of the experiments.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The use of animals in these experiments is required in order to understand how animals perceive and react to different tastes, which are fundamental processes that cannot currently be understood using other techniques. The questions that are asked can be best answered using animal models because increasing stress levels, or inducing anxiety or depression, would be considered unethical in humans. In addition, in order to test if some of our findings can be applied to measure animal welfare, we need to use laboratory species, such as mice and rats.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will ensure that we use the minimum numbers of animals by statistically checking that we have enough animals to test our hypotheses whilst at the same time minimizing numbers. We will use statistical analysis (such as power analysis) based on our previous data, that will identify the optimal number of replicates required to on one hand minimise the number of animals used but on the other hand maximise expected effects to be observed.

Refinement

Explain the choice of animals 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.

Refinement

Rats and mice have been chosen because we want to apply the results of our studies to improve the welfare of these species when they are used in other research projects. They are also important models for understanding the interplay between body and mind in mammals such as rodents and man. Most of the previous work in this field has been carried out in rodents, so our experiments will be based upon previous findings, which reduces the need for preliminary work and increases the likelihood of success.

Harms to the animals will be minimised by selecting the least invasive means for achieving any experimental goal. We will refine our experiments based on the results we get during the project in order to minimise welfare costs to the animals where possible in consultation with animal support and veterinary staff.

PROJECT 147

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Evaluation of adverse bystander effects of medical devices and drugs
Key Words	In vitro model systems, chemical safety, toxicity of metal ions, safety of medical devices, chemical metabolism
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production

	conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Ths project consists of two related studies:

1. Investigating the safety of medical devices, particularly metallic devices, and understanding the mechanisms responsible for adverse reactions in patients.
2. Optimisation of in vitro toxicity testing systems based on cultured liver cells in order to improve prediction of in vivo drug-induced adverse effects in patients.

Our research focuses on the mechanisms responsible for adverse effects of metallic implant devices, particularly cobalt-chromium alloy metal-on-metal hip implants. Such implants release nanoparticulate metal wear debris into patients' bodies, and this results in elevated cobalt and chromium ions in the blood. Mounting evidence has implicated cobalt ions in systemic adverse effects such as cardiac and brain abnormalities in patients with MoM implants, and we will investigate these in vitro in cardiac fibroblasts and in vivo by administration of cobalt to rats.

Adverse reactions to drugs is often related to metabolism to a reactive metabolite, and results in considerable attrition in development of

new drugs, and in withdrawal of drugs from the market. The liver is the main organ responsible for the metabolism of chemicals, and hepatocytes the main cell type in the liver where these reactions take place. Rat liver cells are used, in suspension and in culture, to profile the metabolism and toxicity of chemicals, including new candidate pharmaceuticals. Traditionally cell culture has been carried out on stiff polystyrene Petri dishes, but in our work hepatocyte functions in vitro will be

optimised by matching the mechanical properties of cell culture scaffolds to the mechanical environment of the naturalliver.This will enable us to improve in vitro toxicity testing systems and improve prediction of in vivo drug adverse effects.

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

Adverse drug reactions are a considerable health problem and present a huge impediment to drug development. Our collaborative work with drug discovery teams leads to understanding of the relationship between structure of a drug and its metabolism and toxicity, and permits development of compounds with appropriate bioavailability and minimal toxicity. Medical device safety is of prime concern not only to patients but also to device manufacturers and clinicians: adverse effects can have huge economic significance on several fronts – for example, to the manufacturer and to patients, the latter from both a personal healthcare level and in terms of NHS costs. Our work on safety of medical devices will find out what the targets of cobalt are in the heart and brain, how the ions enter these organs, and investigate mechanisms of toxicity to provide safer medical devices, and help to minimise release of metal ions into patients' bodies.

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

400 adult rats over 5 years

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

The procedure for isolating liver cells is carried out under terminal anaesthesia and is classed as mild. Where chemicals are being administered to animals the procedures are classified as moderate. For adverse effects related to dosing routes, and volumes, local guidelines will be used. Administration of test substances will be based on doses described in the literature. The dose, even in studies designed to investigate mechanisms of toxicity, will be chosen so as to cause no overt symptoms in the animals. However, even with sub-toxic doses there will be inter-animal variation in responses and there is the possibility that adverse reactions will occasionally occur in these experiments (<0.5%). Any indication that an animal is experiencing pain, distress or discomfort will result in the animal being immediately humanely killed. At the end of all experiments animals will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Although metabolism and toxicity studies can be carried out on human tissue, there are several reasons why this is difficult. The liver enzymes and their regulatory pathways are unstable post-mortem, and it is difficult to obtain fresh human liver. Some adverse reactions are only apparent in a few susceptible individuals and it is unlikely that tissue from a susceptible individual would be available. Animal models allow us to manipulate the conditions used for incubations with chemicals to simulate unusual individual patient circumstances.

We have investigated the use of established liver cell lines, for example Hep G2 cells (from a human hepatoma), but the levels of chemical metabolising enzyme activities in these cells are extremely low, and it is not therefore possible to determine the metabolic profiles of chemicals using these cells. Cryopreservation techniques are not suitable for hepatocytes, as they are big cells with slow membrane water transport properties, and are damaged on freezing. We will use HepaRG cells, a hepatoma cell type with reported hepatocyte functions for some metabolic studies, and culture development work.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Each rat yields approximately 400 million liver cells. Incubations in suspension are carried out at 2 x 10^6 cells/ml and in culture at approximately 1.2 x 10^5 cells/cm^2. This enables us to carry out over 100 separate culture experiments or evaluation of the metabolism and toxicity of 15-20 test compounds and is a significant reduction in the number of animal experiments previously used to collect data on metabolism/toxicity of chemicals.

Neuronal and astrocytoma cells have been used over the past year to collect data on uptake and toxicity of Co into CNS cells, and this will be extended to include primary cultures of neuronal cells. Dissemination of Co in vivo will be characterised in rats, and adverse effects will be characterised by several approaches. Ultrasound of the heart informs on parameters of cardiac function, tissues are taken for Western blotting, and for isolation of mRNA, and expression of genes. From each organ of interest 4 samples are taken for mRNA isolation (collected in RNAlater), 3 samples for protein expression by immunoblotting (frozen at -80^{o}C), so that each animal yields 3 results per parameter, maximising the amount of data that can be collected per animal. Each of our time points for investigating the effects of cobalt will have data from 6 rats. This is based upon our previous experience of investigating similar parameters of heart function. Using a statistician's advice animal numbers of n=6 will be used for pilot experiments where effects of cobalt on hypertrophic phenotype are to be examined. Although we do not know the mechanism of cobalt induced

cardiac hypertrophy we anticipate that the effects should be detectable with the same number of animals, and we will collect tissue samples from several organs in the same experiment for storage either in liquid nitrogen or at minus 80 degrees to carry out subsequent immunoblotting for protein expression and isolation of RNA for gene expression.

Refinement

Explain the choice of animals 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.

Refinement

Rats are the lowest vertebrate group for which there is an extensive literature base on the metabolism and toxicity of chemicals. This gives us a wide knowledge base with which to interpret our data.

The majority of the animals used in our projects are used under a mild severity band and the invasive procedure for isolation of the liver cells is carried out under terminal anaesthesia. Animals on the moderate protocol are only kept for short periods of time after dosing . We believe that these are the least severe procedures that would yield satisfactory results.

PROJECT 148

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Breeding and maintenance of genetically altered mice
Key Words	Transgenic, Mouse, Breeding
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To breed and maintain genetically altered animals to be used in scientific research to help understand mechanisms of 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)?

Genetically altered mice are valuable animal models that contribute to the elucidation of a wide range of biological processes and diseases like cancer. Although in vitro approaches provide critical data, the use of animal models is essential to understand the very complex scenario of this disease.

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

Mice only. In order to produce sufficient mouse numbers of required genotype for use in experiments, it is expected that approximately 25,000 mice will need to be bred and maintained annually.

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?

Genetically altered animals will be maintained under this licence in order to understand further different processes associate with cancer. The various steps involved will be: 1: Injection of hormone to increase egg production in female mice. 2: Female mice may have embryos implanted. 3: Vasectomy of male mice to allow them to be used to make females have phantom pregnancies and make them ready for receiving embryos generated in other females. Each new strain generated will have a very well described expected profile. However animals will be monitored for unpredicted adverse effects and profiles will be monitored. Surgical Procedures will be performed under anaesthesia and using pain relief and following aseptic methods

to minimize risk of post-surgical complications. Anaesthesia will be carefully and regularly monitored to ensure that an adequate depth is maintained throughout any surgical procedure. Mice will be monitored regularly for their health status throughout all procedures. All procedures will be undertaken by trained, competent people. Mice that are no longer going to be used will be humanely killed following the accepted protocol. Animals that are fully recovered at the end of procedures may be kept alive at the establishment (with the agreement of a veterinary surgeon), with a view to their re-use on procedures if appropriate and licensed. No mice with genetic disabilities exceeding moderate severity will be bred on this licence.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The different animal models maintained and bred under this license will integrate the complete range of molecular, cellular, physiological and behavioural interactions necessary to fully understand how genetic modifications result in normal or abnormal processes, focusing on cancer.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Breeding programmes will be agreed in advance and regularly reviewed to optimally meet anticipated demand. Breeding programmes will be optimised wherever possible to produce only the required genotype.

Freezing of eggs / embryos and sperm will be carried as routine. Archiving of lines will avoid wastage from the need to maintain colonies by continuous breeding.

Refinement

Explain the choice of animals 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.

Refinement

Researchers have studied laboratory mice as models of human cancer for many years. Their use as cancer models has provided exceptional insight into the biology and genetics of human cancers. There are standard protocols, methods and

reagents used that have been optimised for manipulation of genes in this species and their acknowledged benefits for use.

The mice will be cared for by dedicated, experienced animal technologists who have the expertise and skills required to breed mice. Welfare problems that may occur at an early stage will be monitored carefully to determine appropriate end points in consultation with experienced animal husbandry technicians and veterinary surgeons.

PROJECT 149

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	INVESTIGATION INTO THE BIOLOGY AND THERAPY OF NEUROMUSCULAR DISEASE
Key Words	neuromuscular disease, translational research, drug delivery
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Our research is focused on developing therapies for neurological and neuromuscular disorders. At least 70,000 children and adults live with a neuromuscular condition in the UK. For the majority of these disorders there is no cure. The most promising therapeutic approach for many of these disorders is to target the genetic defect underlying the disease. However, it is very difficult to target these therapies towards the skeletal muscle, heart, and brain where they are needed.

We have developed to delivery systems by which gene-based therapies are coupled to small delivery molecules, called peptides, or incorporated into small particles, called extracellular vesicles (EVs). Both peptides and EVs act as a shuttle to target the active drugs towards the muscle and brain. We have successfully targeted muscle and brain with both approaches.

We will continue this approach to drug delivery through three aims: (1) investigate new methods for drug delivery to the muscle and brain for neuromuscular disorders (2) develop new forms of gene-based and small molecule therapies for neuromuscular disorders and (3) study the underlying biology of neuromuscular disorders to identify new avenues for drug development. The ultimate goal for all these aims is to develop drugs which are suitable for clinical development.

By combining knowledge of the processes underlying diseases with drug development and improvements in drug delivery, our aim is to translate treatments for neurological and neuromuscular disorders to the clinic as soon as possible.

The Wood lab will continue its work through three aims: (1) investigate new methods for drug delivery to the muscle and brain for neuromuscular disorders (2) develop new forms of gene-based and small molecule therapies for neuromuscular disorders and (3) study the underlying biology of neuromuscular disorders to identify new avenues for drug development. The ultimate goal for all these aims is to develop drugs which are suitable for clinical development.

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

The transition of gene therapies from the lab into the clinic is often stalled by lack of efficient delivery of the drugs to the targeted tissue, namely skeletal muscle, heart and brain. We have generated two modes of drug delivery (peptide and membrane bound vesicles) which efficiently delivers gene therapies to the muscle and brain. Our work had mainly focused on Duchenne muscular dystrophy and spinal muscular atrophy, two neuromuscular disorders without curative therapies. In the course of 2-5 years we expect to take part in at least one phase I/II clinical trial. The work in this project will continue to develop these drugs forward while also developing new therapies for the multiple neuromuscular disorders still left untreated.

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

We expect to use 500-800 mice over the course of a year.

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

The most clinically relevant experimental animal models for the fatal neuromuscular diseases spinal muscular atrophy (SMA) and motor neuron disease (ALS) display adverse phenotypic effects due to the loss of neurons leading to reduced movement, reduction in body weight and early death. These adverse effects are minimised by very close monitoring of the disease progression and selecting appropriate humane endpoints, however, most animals are likely to experience a moderate level of severity. These animal models are particularly important for drug discovery as they accurately mimic many of the clinical features found within patients with SMA and

ALS including disease progression during development. By working with these models we aim to generate new drug therapies for these fatal conditions. While we very carefully introduce new drugs, on the basis of extensive in vitro screens and testing, at low concentrations and closely monitor the animals, such new drugs can occasionally generate unpredictable results and could worsen progression of a disease phenotype instead of improving it. For this reason we designate a severe level of severity for one of our experimental protocols. It is however our strong expectation that less than 2% of total animals used in this licence, including breeding protocols 1 and 2, would experience this level of severity.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Most of our drug development occurs in animal alternatives prior to animal experimentation, namely in silico (computational) and in vitro (cell culture) systems. Computational methods are increasingly sophisticated for the early design of gene-based therapies and are increasingly used to design initial drugs for in vitro testing. The cell culture systems used (including cell model systems derived from patients) subsequently play a crucial role in understanding the mechanism of drug action which allows us to refine the design of new therapies and guide future drug development as well as providing early insight into safety. Less than 10% of the gene-based drugs we evaluate in cultured cells are subsequently tested in animal models and therefore those that are tested in animal models have already undergone extensive in vitro evaluation and selection based on cell activity and safety. Having said that, the goal of our work is to develop new drugs for the treatment of currently untreatable neuromuscular diseases, and therefore we are required to understand drug mechanism of action and effectiveness in the context of the whole body system. While many drugs are found to be active and safe in cultured cell models, the complex nature of animal physiological systems especially the nervous system and including structures such as the blood brain barrier between the circulating blood and the brain itself, complicates the delivery of the large gene-based drugs to the appropriate target organs and cells. Therefore while critically important for drug development, the animal experiments are rarely predictable. However basing these drugs on high quality in silico and in vitro data increases their chances of success. In addition, only in the animal are we truly able to test and understand the safety profile of any new drug. Therefore without the information resulting from well-designed animal experiments we would not be able to enhance the efficacy and understand the safety of new drug therapies, a critical requirement for any new drug to be brought to the clinic and ultimately to benefit patients.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We take precautions to only take drugs successful in cultured cells into the animal models. Variations among groups of animals are minimised by matching age, gender and strains for all treatment groups. All studies begin with a small pilot group. Only drugs successfully active in the small group will be used for larger studies. A pilot study also ensure we use the appropriate number of animals.

Refinement

Explain the choice of animals 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.

Refinement

In most cases we will use genetically altered mice which models relevant diseases both physically and biochemically.

The first time a drug is used in animals it is given at suboptimal dose to ensure tolerability. We then give incrementally higher doses until our optimal dose is reached. Should animals begin to show intolerability to suboptimal doses, that particular drug is no longer used or only used at the tolerated dose.

PROJECT 150

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	THERAPEUTIC TARGETS IN INFLAMMATORY BOWEL DISEASE
Key Words	Inflammatory bowel disease, therapy
Expected duration of the project	3 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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The proposed programme aims to the investigation of much needed new therapeutic applications for chronic inflammatory bowel diseases (IBD). IBD include Crohn's Disease (CD) and Ulcerative Colitis (UC), and affect more than 200,000 individuals in the United Kingdom, and these numbers steadily increase. This increase is most noticeable in developed and developing countries which are adapting a 'westernised' lifestyle and diet. Genetics cannot explain this phenomenon; environmental factors, including diet and toxins, affect the way genes are turned on and off through chemical changes called epigenetic modifications. In addition, current medical treatments for IBD target the patient's defence (immune) system and may be ineffective or often limited by unwanted effects. Thus, the identification of novel therapeutics for IBD patients is of great importance. This project aims to exploit specific epigenetic mechanisms affected in IBD patients for the development of new drug treatments.

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

The identification of novel therapeutic targets in IBD. We will test the effects of new compounds on disease progression. Molecular analyses will reveal new mechanisms regulating disease development. We will address the value and benefits of treatments that do not target exclusively or directly the patient's immune system, an approach that comes with several limitations. This study encompasses analyses of both the efficiency and safety of new drugs that can be rapidly brought to the clinic.

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

It is estimated that up to 430 wild type and genetically altered mice will be used for this project over a 3-year period.

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?

Two models of colitis will be developed. One will involve administering in the drinking water a chemical (Dextran Sulphate Sodium Salt, DSS), and the other will involve the transfer of immune cells (T cells) by intraperitoneal injection. The signs of colon inflammation in these models are similar to the ones observed in patients with IBD and include weight loss, blood in stools or diarrhoea. Intracolonic administration of specific medicines will be applied to assess their ability to inhibit disease. This approach resembles a route of drug administration commonly used in patients (enema). All protocols have been designed to achieve the desired objectives without compromising the animal's welfare. Adverse effects such as transient discomfort from injections, and weight loss, slightly loose stool and slight presence of blood in the colon are expected. For some of the animals (control groups), severity will be mild. Cumulative severity will be moderate. Candidate medicines will be administered intracolonically at low volumes to anaesthetised mice, at the lowest possible therapeutic concentration, in order minimise potential side effects. The overall severity of the project is expected to be 'Moderate', and all animals will be culled at the end of each study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The requirement to use animal models stems from the fact that IBD is a multifactorial disease, and impossible to replace with other approaches. The pathogenesis involves host-microbiome interactions, cell-cell interactions, activation of inflammatory cells, loss of epithelial integrity and mucosal homeostasis. The extensive research data already available in mouse models, as well as the availability of inbred strains, offers an excellent model for studying human diseases. Compared to other mammals, IBD develops in a short timeframe in mice and it is based on well-established protocols. A phylogenetically lower species cannot replace the use of mice. Mouse models are important in this study, as *in vitro* or *in silico* assays, to recapitulate the human IBD are completely missing.

For the identification of the substances to be tested in mice, we have employed cell-based assays. By employing *in vitro* assays, we replace a large number of mice that would be required to test the whole range of compounds.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will analyse the effects of the tested substances on functional endpoints relevant to IBD, the inflammation and mucosal healing *in vitro*. Molecular analyses will address the most efficient concentration of the candidate medicines with the minimum toxic effects. Thus, the use of mice for titration of compound doses will be avoided or significantly reduced.

Upon completion of the experiments, mouse tissues and fluids will be collected to verify the lack of toxic effects. Combination of the evaluation of drug efficiency with the analysis of toxicity in the same animals, further reduces the number of mice used.

The variation between individual mice and the variable development of the disease has been taken into consideration in order to ensure the delivery of valid findings. Experiments have been designed to include the minimum possible number of mice needed in order to reach statistical significance in the anticipated results.

Refinement

Explain the choice of animals 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.

Refinement

This study will employ two different mouse models of IBD, DSS and T cell transfer. We have selected these mouse models for our studies following the screening of multiple models of IBD because they reproduce the mechanisms observed in patients with colitis. Chemically-induced colitis (with DSS) recapitulates the loss of epithelial cell barrier integrity, whereas the immune cell transfer-induced model (transfer of T cells) recapitulates the persistent aggressive inflammatory response, both of these elements are the major host-related pathogenetic mechanisms which appear to drive human disease.

Both models are considered essential. First, because they reproduce the mechanisms identified in patients, and second, because this study aims to formulate the design of a clinical trial and should encompass the concept of variability observed in human disease. Notably, it is now widely accepted that to assess the clinical efficacy of a drug, its ability to reverse disease in at least two different animal models of chronic intestinal inflammation is required.

A pilot study will be used to assess the exact time frame of disease development. This would allow the application of treatments within specified time limits to exclude the possibility of increased severity. The protocols employed are well established. Every effort will be made to reduce and relieve pain in the mice. We have developed a protocol for the intracolonic delivery of therapeutics in order to increase the efficiency and minimise potential systemic side effects. The protocols employed are well established. Male mice are selected for the DSS model due to reproducibility and susceptibility, and female animals will be used for the T cell model because they are less aggressive than males. This will avoid the negative impact of stress and wounds.

In the DSS model, upon each cycle of DSS administration animals experience gradual weight loss and reduced stool consistency. Progressively, after the third cycle animals may present diarrhoea and bloody stools. The clinical symptoms should not exceed moderate discomfort and between cycles animals are expected to recover. In the T cell transfer model, the signs of colitis develop after three weeks as slow progressive weight loss which is later accompanied by loose stools and diarrhoea. Because disease is gradually worsening we aim to perform experiments upon detection of early signs of inflammation, and if increased weight loss, rectal bleeding and diarrhoea are observed they will be terminated.

Every effort will be made to reduce and relief the pain of the mice. Animals will be kept for the minimum possible time outside the cages for weighing, observation and administration of therapeutics. We have developed a protocol for the intracolonic delivery of candidate therapeutics in a small volume in order to increase the efficiency in directly targeting the diseased tissue and minimise potential systemic side-effects. Candidate medicines will be used at their lowest therapeutic concentration and dose volume to minimise discomfort. When required, injections will be performed according to established methods and needle sizes. We expect that the candidate medicines will have no, or only minor, adverse effects. Mice will be monitored daily and at least twice per day (more often if indicated) when disease develops for pain/distress and if they reach the humane endpoint indicated by weight loss of 15%, they will be culled before the study's scientific endpoint. If any welfare concerns arise, then the named animal care and welfare officer and/or the named veterinary surgeon will be consulted.

PROJECT 151

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Radiolabelled molecules for cancer imaging and therapy
Key Words	radioisotope, Cancer, targeted radiotherapy, chemotherapy, imaging
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project has 3 parts: 1) Optimisation of a technique (targeted radiotherapy (TR)) delivering radioactive molecules to cancers to destroy them (2) Characterisation of novel radiolabelled probes that can be used to detect cancer (3) Measure the efficacy of anticancer drug combinations and the effect of treatment on the uptake of radiotracers used in medical imaging.

1) Cancer patients often present with metastatic disease where cancer cells have spread from the primary cancer to establish new cancers elsewhere in the body. Whilst radiotherapy is the most effective anticancer treatment for cancers that cannot be surgically removed it cannot be used to control metastasis. Currently chemotherapy drugs, which distribute around the body, are used to treat metastases but cancers and their metastases almost always develop resistance to chemotherapy. Targeted radiotherapy (TR) involves injecting radioactive drugs targeted to cancer cells which seek out primary tumours and metastases and use their radioactive payload to locally irradiate cancer tissue. In this project we will produce molecules capable of delivering the necessary radiation dose to tumours and their metastases. They will be tested on biological models for efficacy and safety. Results from this study will provide requisite data for a full clinical study.

2) Early cancer detection is critical to long term survival. Positron emission tomography (PET) is the most sensitive medical imaging technique for cancer detection. Currently patients are administered with a radioactive tracer called FDG which is concentrated by cancers facilitating detection of the cancer in the body by the PET camera. Although FDG is very useful its uptake is not restricted to cancer so novel cancer imaging agents are under development. These need to be tested to

determine their cancer targeting potential and suitability with respect to where they locate in the body and how long they stay in the body.

3) Combinations of drugs can increase the anticancer effect compared with giving a single drug but cancer response varies between patients. When a cancer is responding to drug treatment, the uptake of a radiotracer by the cancer tends to decrease (this is measured in patients using a PET camera). Here we will test how combination treatments modify the uptake a radioactive tracer to justify using PET in patients to detect response to drug combinations.

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

1) The anticancer efficiency of targeted radiotherapy (TR) is limited by the poor distribution of the TR molecules within cancers due to regions in cancers where blood flow is poor and due to variation in the amount of TR-target that different cancer cells display. To overcome these problems this project will determine the optimum chemical makeup of the TR molecules. This optimisation will provide the most suitable molecules for a subsequent clinical study. 2) Medical imaging techniques including PET which detects radioactive molecules (tracers) that are attracted to cancers are proving to be very useful in cancer detection and in identifying response to treatment. Novel tracers are being developed which are attracted to cancers but less so to non-cancer tissue to more precisely identify cancers and so reduce uncertainty in cancer diagnosis. 3) Changes in the uptake of tracers during treatment (compared with before treatment) can signal response or non-response at an early time point. Where patients are shown not to be responding to a treatment combination it can be changed to a more effective one.

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

1) Project 1 should take 2-3 years and will use about 250 mice in order to optimise our targeted radiotherapy molecules. 2) Project 2 will be ongoing as new tracers are produced (maximum of 4). Each study will need 50 mice (total of 200). 3) Project 3 will examine treatment efficacy and the effect of treatment on the uptake of the tracer and require 200 mice. This study will take 2-3 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?

Tumours (cancers) will be induced in some mice by subcutaneous (under the skin) injection of cancer cells into the flank. Initial injection may be associated with some inflammation. Tumours will be grown to a maximum size of 15mm (longest dimension) which will not hinder movement nor will they spread as the animals will be humanely killed after a few weeks. This type of procedure is generally assigned a moderate level of severity. Some animals will be injected with clinically relevant

doses of radioactive molecules that target the cancers and are not expected to have any adverse effects. Some mice will receive anticancer drugs that target signalling pathways in cells and are not expected to exhibit any side effects (mild level of severity). At the end of each experiment mice will be killed by a humane method and tissues collected.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

1) NC3Rs website has been examined for possible cancer models that could replace animals. A 3D model that is less than 1mm in diameter was found. However we need ones that are 10-15mm in length as we need to demonstrate that the targeted radiotherapy molecules distribute within a solid tumour and produce a uniform dose distribution throughout. The dose is deposited (killing cancer cells) up to 10mm away from the decaying nuclide so the tumours need to be 10-15mm in length to facilitate uptake within a cell kill range. This is in common with comparable studies in the literature. No alternative models for the development of targeted radiotherapy molecules could be found.

2) To determine how a novel imaging agent distributes within the body, how long it stays in the circulation and its excretory route are essential information before a novel cancer imaging agent would be allowed to be used in the imaging of a patient. To determine how the uptake of tracers is influenced by treatment response is initially tested in vitro using isolated cancer cells. However in vivo many other factors influence what happens to the tracer so *in vitro* findings need to be verified *in vivo* prior to clinical translation.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

1) *In vitro* studies using cells grown in culture can be used to determine the sensitivity of cancer cells to types of cytotoxic radionuclide and to drugs. We will use these methods to inform on the levels of anticancer agents likely to cause tumour regression. Pilot studies based on these findings and the literature for comparable studies will reduce the number of animals required to determine sensitive anticancer agent doses.

2) To ensure that the *in vivo* studies are justified tracers will be screened for specific binding to cancer cells before they are tested *in vivo*.

Refinement

Explain the choice of animals 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.

Refinement

Xenograft (cancers derived from human cancers and grown in animals) cancer models are grown in immunocompromised mice. The mice will be frequently checked to ensure that injections of cancer cells, anticancer drugs, radioactive tracers do not produce any unexpected adverse effects. Formation of a lump within 24h after injection or increased licking of the injection site would give an initial indication of an (unlikely) adverse effect. The animals would be expected to show no adverse effects but would be closely monitored for changes in appearance.

At the end of procedures the animals are humanely killed and tissues analysed

For some of the studies we will require a large blood sample which can most humanely be acquired by cardiac puncture under terminal anaesthesia.

Where oral gavage is used to administer drugs flexible tubing will be used to decrease the discomfort experienced by the animal.

PROJECT 152

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Novel Therapies for Severe Bacterial Infections
Key Words	Bacteria, Infection
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Severe bacterial infections remain a threat to human health. In recent years, there has been an inexorable rise in marked antibiotic resistance within bacteria. Such drug-resistant infections can be very difficult to treat and in some cases no current effective therapies remain. There is thus an urgent need to develop novel therapies for bacterial infections. This project will explore a number of avenues that we believe will offer new therapies for bacterial infections. We will exploit natural antibiotics produced by bacteria, called bacteriocins, to establish if they can be used to treat bacterial infections. We will define key elements of the body's natural defences to these infections, to develop new therapies that augment these responses that may then prevent or ameliorate infection.

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 outcome of these studies will potentially lead to novel therapeutic interventions for severe human bacterial infections.

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

We are using mice in this project and estimate to use about 4500 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 levels of severity? What will happen to the animals at the end?

The very nature of bacterial infection does mean that some of the models we use are severe in nature. The effects are on general characteristics, such as coat condition, movement and posture. Additionally, in animals infected by the respiratory route, they may show signs of laboured breathing. However, we have a very clear and well-

defined monitoring process that ensures animals are humanely killed once their clinical condition has reached a pre-defined level of severity. At this point, animals will be removed from the study and humanely killed. Otherwise, at the end of the defined time points in the protocols, the animals will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The studies described in this licence cannot be done without the use of live animals. Extensive laboratory experiments outside of living organisms ("*in vitro*") analysis is used to identify those experiments that can only be done using whole animals. The animal experiments are designed to study the host/parasite relationship using bacterial pathogens in animal models of human disease. These studies can only be done in systems with an intact immune system, vascular supply etc. To do this an infectious dose of bacteria is administered intended to produce clinical disease without overwhelming the animal. The pathogenesis of the disease can then be studied by serial killing during its course. Analysis of the infectious process by bacteriology, immunology, histology, imaging and the use of bacterial and animal mutants allows new therapeutic and vaccine strategies to be investigated. Good reagents for studying these processes and genetically modified mice are available to study response to infection in animal models. Use will be made of *in vitro* models where simple cellular interactions between cells will be studied. However, the complex interplay between bacterial pathogens and the immune system can only be effectively studied with the use of live animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The dose range of novel reagents will be estimated initially using *in vitro* experiments, thus reducing the numbers of animals required to establish effective doses. Our experimental design has been carefully planned to use the smallest numbers of animals required to give statistically meaningful results. For example, where possible, a control group will be used as a comparator between several experimental arms to minimize animal use. Where exact effective doses of novel therapies are required, we have designed a protocol that will evaluate the results from all doses at the same time, which can then be manipulated mathematically, reducing the numbers of animals required.

Refinement

Explain the choice of animals 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.

Refinement

The mice models we employ are very good models of human infection with the pathogens we study and are very amenable to further experimental study because of the wealth of different reagents available for this species as well as the ability to use well-defined genetically modified animals. We have developed robust criteria for evaluation of animal well-being during the course of the experiments which allow the experiments to be terminated when these reach pre-determined levels. Staff employed on the project will monitor animals intensively following infection, as will the dedicated staff within the animal care facility. We have extensive prior experience of the models to be employed and have been able to intervene effectively to remove animals from studies where their clinical condition has exceed a set limit, thus reducing the potential animal suffering. We will also employ where possible remote monitoring techniques, such as imaging of live bacteria within the animals that can provide a guide to the progress of infection without requiring animals to be humanely killed.

PROJECT 153

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Development of Bacteriotherapies to Treat Intestinal Dysbiosis
Key Words	Bacteriotherapy, microbiota, dysbiosis, infection, inflammatory bowel disease
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Mammals are colonised by diverse and abundant microbial communities, termed *microbiota*, required for normal immune system development, sustenance and resistance to pathogens. Pathological imbalances in the composition of the microbial communities, termed dysbiosis, are associated with or cause a range of significant phenotypes, diseases and poorly understood syndromes, such as IBD and infection susceptibility. The aims of the project are to design and test bacteriotherapies - defined mixtures of beneficial bacteria - to correct dysbiosis and treat Clostridium difficile infection and Intestinal Bowel Disease (IBD) in murine disease models.

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 significant shorter term output of programme of work will be to generate data to demonstrate bacteriotherapies that can be used to progress to pre-clinical development in preparation for clinical studies in humans. In the longer term, the work outlined in this proposal is expected to lead to novel bacteriotherapies to treat intestinal dysbiosis linked to Clostridium difficile infection and Intestinal Bowel Disease (IBD) in humans to reduce morbidity and mortality. The results of the research will be published in scientific journals and presented at scientific conferences. New mouse models may be patented and shared with other researchers.

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

Over the 5 year period of the project, we anticipate to use 6000 mice.

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

Animals are expected to experience mild to moderate intestinal disease and inflammation and will be treated with bacteriotherapies with the goal of curing disease. Mice will be humanely killed at the end of the experiments.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The complex environment of mammalian surfaces and organs cannot be accurately modelled *in vitro* and, as a result, the immune response and pathological features linked to host-microbe interactions cannot be recapitulated *in vitro.* However, some aspects of host-microbe interactions can be modelled in vitro, such as competition experiments on nutrient agar plates, and we continuously consider their use and development in light of new experimental data. We carry out microbiological culturing, look at DNA and proteins, and grow cells such as human or mouse derived intestine cells in culture, to help us identify the bacterial genes we need to study, before we move on to using mice.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have established infection protocols with reproducible disease courses and defined outcomes using pathogens with clinical features that are relevant to human diseases. During this period we have also established statistical measures linked to biologically relevant outcomes to ensure we employ the minimal number of animals per experiment.

The results of the research will be published in scientific journals and presented at scientific conferences.

New mouse models may be patented and shared with other researchers.

Refinement

Explain the choice of animals 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.

Refinement

We use mice for select experiments as they represent an ideal model to study host-microbe interactions, and serve as an invaluable pre-clinical model for therapy development. Mice can be genetically manipulated to mutate genes relevant to human disease susceptibility and there are immunological reagents available to monitor the host response to microbe interactions. We also closely monitor mice on a daily basis for signs of illness and suffering, scoring for physical signs of illness such as piloerection, hunched gait and mobility alongwith weight loss. Our animal facility uses a sophisticated database to track the health status of every animal.

PROJECT 154

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The role of TRP ion channels in pain
Key Words	Pain, analgesia, cold, arthritid, ion channels, G proteins
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Pain is a common medical condition associated with many diseases such as arthritis, and poses significant challenge for everyday life of the patients. Cold is widely used as an analgesic for alleviating pain. Paradoxically, cold also triggers pain. The opposite effects of cold may be the reason why the cold therapy has only a modest effect. However, it is poorly understood how cold exerts such contrasting effects on pain. The objectives of this project are: (1) to determine sensory nerve cells responsible for transmitting pain and analgesia, respectively, evoked by cold; (2) to determine the role of these nerve cells in chronic pain. This research could reveal novel pain and analgesic pathways leading to more effective pain 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)?

Scientifically, the project could reveal two novel nerve circuits responsible for carrying pain and analgesia, respectively. It would thus advance our understanding of the opposing effects of cold on pain. Practically, the project could act as a fundamental basis for guiding and improving the current practice of cold therapy. Finally, in the long term, the project could lead to the development of novel analgesics that target on the pain pathways revealed in this project. The project could thus be beneficial to many of the patients suffering from pain.

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

Mice will be used. It is estimated that roughly 1600 mice will be used over five years of time.

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

We will induce arthritis pain in mice followed by assessing pain behaviours of mice. Therefore, mice would inevitably experience moderate severity of knee pain and have difficulty in free-walking. All the animals will be culled at the end of experiments using a Schedule 1 method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The main objective of the project is to test to what extent the identified nerve cells contribute to pain and analgesia in animals. The research thus necessitates the use of whole animals for assessing systemic pain behavioural responses. Cell and tissue models cannot be used to predict or synthesize systemic pain behaviours, and thus cannot be used as a replacement, though we examine functions of isolated cells to predict animal behaviours

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Power calculations will be used to estimate the minimal number of animals required for experiments based on literature and previous experience. Furthermore, unbiased experimental design will be carried out to minimize the number of animals to be used, while obtaining more information.

Refinement

Explain the choice of animals 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.

Refinement

Species of animals:

Mice were chosen for the following reasons: first, pain behaviours of mice are well characterized. It will make our results comparable to the results obtained from other labs. Secondly, the transgenic mice lines to be used in the project are already

available, thus obviating the need to generate transgenic lines from a different species from scratch.

Animal models:

To examine the specific effect of cold on arthritis pain, acute arthritis pain and chronic osteoarthritis pain models were chosen. These pain models are comparable to arthritis pain in humans and are thus representative, and have been widely used to study pain mechanisms. Moreover, arthritis pain in these models can be conveniently induced in one knee of mice, while another knee can acts as a control, thereby capable of reducing the overall suffering of animals. We will aim to reduce the duration of arthritis pain to less than 4 weeks in order to minimize pain suffering. However, analgesics cannot be given to animals during arthritis pain, because it is our primary objective to monitor the effect of cold on pain behaviours.

Measures to minimise welfare costs:

All the pain assessment will be carried out aiming to reduce the exposure time of mice to painful stimuli and to avoid tissue injury. Drug administration will be carried out in accordance with the LASA guidelines. Animals with signs of ill-health will be humanely culled using a Schedule 1 method.

PROJECT 155

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Molecular mechanisms controlling Salt and Potassium homeostasis
Key Words	Hypertension (high blood pressure), kidney, dietary salt
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Hypertension (high blood pressure) is a major public health problem affecting more than a billion people worldwide with complications including stroke, heart failure and kidney failure. It is most frequently caused by inappropriate salt retention by the kidney. We have learnt a lot about the mechanisms in the kidney for regulating the balance of salt loss and salt retention through the study of rare inherited forms of blood pressure. One of these (Gordon Syndrome or FHHt) causes salt retention by activating a specific salt transporter in the kidney called NCC that is blocked by thiazide diuretics (probably the most widely used blood pressure lowering drugs world-wide). A second group of inherited syndromes (and also the commonest non-inherited and potentially curable forms of hypertension) cause salt retention by causing the adrenal gland to secrete excessive amounts of aldosterone, which is the hormone controlling salt retention in the kidney. In addition, around 10% of essential human hypertension is thought to be caused by excess aldosterone production and in a third of cases it derives from small benign nodules in the adrenal glands. These offer the prospect of a potential long-term cure through removal of the affected adrenal gland by key-hole surgery. Yet, there are still significant gaps in our understanding of how NCC is regulated and what drives the adrenal gland to over-secrete aldosterone. This project will address both these issues.

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 pathway regulating the NCC protein in the kidney can be potentially blocked by small chemical molecules (drugs). This would allow us to generate new drugs to act as diuretics ('water tablets') and lower blood pressure. A better understanding of the adrenal causes of hypertension may similarly enable new drugs to be developed

and/or allow better understanding of the processes driving the formation of the small benign tumours in the adrenal gland (which are often the source of the excess aldosterone driving the blood pressure).

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

The project will only use mice and may use up to 3550 mice in its 5-year duration.

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

We will breed some genetically modified mice to mimic the genetic changes seen in human hypertension syndromes. These do not have an adverse effect on the mice except for causing hypertension. Most of the procedures done on these genetically modified mice are mild and wherever possible are non-invasive (eg the measurement of the blood pressure from a tail BP cuff or cardiac function by surface Doppler imaging as in human ECHO tests). All animals will be euthanised.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Blood pressure and other measures of kidney function can only be observed in a whole living animal. However, in using the mouse as a model of human genetic hypertension we will generate the mice by modern gene editing methods (CRISPR), which represents the most efficent method in terms of minimising the numbers of animals needed. We will also explore the functions of the human mutations we are interested in tissue culture and other cell based systems before exploring them in a mouse. This will allow us to explore a lot of the biology of the human mutations before we look at their eefect on the BP and kidney function.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We are very keen to reduce the numbers of mice used. Hence, all of our experiments will be designed to ensure they have adequate (but not execessive) statistical power. So that if there is a biological effect in the mice we will be able to detect it - that is to minimise the risk of a false negative result. The experiments will also use randomisation and blinding to ensure that the results are as robust and

reproducible as possible. With these safeguards, we will use the minimum number of animals to observe the effects we are looking for with an appropraite level of precision.

Refinement

Explain the choice of animals 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.

Refinement

In this project we will be using where ever possible non-invasive methods of measure the BP of the mice and the performance of their hearts. This will involve using tail-cuff measurements (as used in the human measurement of BP) and heart ECHO cardiograms (again as used in humans and involves simply bouncing sound waves off the heart.

PROJECT 156

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanisms of Immunoregulation
Key Words	Immune response, Inflammation, Arthritis, Cardiovascular disease, Scleroderma, Influenza, Malaria
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The purpose of this licence is to identify the critical cellular and molecular interactions that underlie the induction of immune responses and inflammation *in vivo*. This information will aid the rational development of new vaccines for use in prevention of infectious disease as well as the development of new therapies to treat inflammatory diseases such as rheumatoid arthritis and atherosclerosis.

Many of the agents used to treat inflammatory diseases have been identified through empirical approaches. This is largely because we do not understand precisely the cellular and molecular interactions involved in the initiation, maintenance and regulation of the immune response. Consequently, we do not know how, when and where a potential immunotherapeutic agent could influence these interactions, particularly as it has been difficult up until now to track cells of the immune system in living animals. As there remains an unmet clinical need for new and improved treatments, this type of information is essential for the rational development of new vaccines and immunotherapeutic agents.

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

Work carried out in this project will identify the important cellular and molecular interactions underlying inflammatory and infectious diseases. These studies will show how, when and where these interactions occur in the immune system and identify interactions between different cell types which drive the immune-inflammatory response. This information is of fundamental scientific importance, will identify how existing therapeutic agents work and importantly reveal new therapeutic targets for drug development. All the information gathered from such studies will

assist the biomedical community to design better drugs, modify existing drugs and apply them more rationally. This will bring benefit to patients in disease areas where there is an unmet clinical need.

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

All animals to be used in these studies will be mice as they are the lowest vertebrates on the evolutionary scale in which suitable models to study the role of the immune system in infectious and inflammatory disease states have been developed. Furthermore, the genetically modified models that we use to refine and reduce the application of inflammatory disease models have only been developed in mice. We estimate to use around 30,000 mice over the 5-year duration of the project.

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

The immune system is highly complex and dynamic with cellular interactions only occurring in specific physiological niches and environments. The use of animal models is ultimately required to produce predictive results relevant to complex multifactorial human diseases such as arthritis, atherosclerosis, Sjögren's syndrome, malaria and influenza. Anaesthetised and conscious animals will be used in these studies and surgical techniques will be kept to a minimum. Any surgical techniques will be carried out with general anaesthesia. Where appropriate analgesia will be used. All animals will be closely monitored. Any animal displaying deviation from normal health, other than due to the inevitable effects of the procedure, will be promptly euthanized or withdrawn from the procedure and referred for veterinary attention. The procedures will be kept to the minimum duration possible. For each ‘severe’ protocol, specific monitoring and scoring systems will be used. These will be reassessed and reviewed throughout the study. Up to date guidance will be monitored via the NC3Rs website (http://www.nc3rs.org.uk/). All animals will be euthanized at the end of the experiment.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Although *in vitro* studies can recapitulate some aspects of the interactions between immune system cells, they cannot adequately model the complete and complex array of immune responses involved in the generation of protective and pathological responses. Such multifaceted interactions between the adaptive immune system with host organs/infectious agents underpinning pathological or protective immune

responses cannot be fully modelled *in vitro*. In addition, not all infectious agents can be produced or maintained *in vitro*. Therefore, further *in vivo* work is required.

Mice are used in this project as they represent the lowest vertebrate group where models of infection, rheumatoid arthritis, lung and salivary gland inflammation, skin fibrosis and cardiovascular disease have been developed. Moreover, the transgenic and knockout models, which will enable our delineation of such processes, are only available in mice.

In vitro studies will be employed where possible, having a variety of collaborators with whom we regularly discuss the potential options for replacement, working on organoid culture approaches and *in silico* models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

A balance between keeping animal numbers to a minimum and achieving statistical validity in experiments has to be achieved. Previous extensive experience has allowed us to ensure that animal usage in our studies is kept to a minimum and each experiment is carefully designed to ensure the minimum use of animals necessary to achieve our scientific aims.

Each protocol is based on extensive experience and has been optimized to ensure minimal suffering for the animals involved. Before starting experiments we use calculations that allow us to work out the minimal number of animals required for us to observe the expected difference (power calculation). In designing new experiments, statistical advice on experimental design will be sought if necessary [REDACTED].

We have developed protocols [REDACTED]that allow us to monitor disease onset in a minimally invasive fashion. Repeated imaging of the same animal can be carried out not only improving the quality of our data but also reducing the number of animals required for temporal studies.

[REDACTED]

Refinement

Explain the choice of animals 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.

Refinement

Animals brought in from accredited breeders etc. are given a period of acclimatization and handling before use experimentally. Our models of infection and inflammatory disease have been developed and refined and give repeatable results of low variability and high quality. The models have also been refined to reduce suffering to the minimum levels likely to give satisfactory result. Where procedures are known to cause adverse effects the method using the least severe adverse effect will be used initially. Animals will be given soft bedding and soft food to alleviate suffering where appropriate.

The endpoints described (see Appendix 1) have been established such that veterinary opinion should not be required before animals are euthanized. However, any animals displaying deviation from normal health, other than due to the inevitable effects of the procedure, will be promptly euthanased or withdrawn from the procedure and referred for veterinary attention. The procedures will be kept to the minimum duration possible.

For each ‘severe’ protocol, specific monitoring and scoring systems will be used. These will be reassessed and reviewed throughout the study. Up to date guidance will be monitored via the NC3Rs website (http://www.nc3rs.org.uk/).

In addition, we have developed protocols using *in vivo* imaging systems that allow us to monitor disease onset in a minimally invasive fashion and at earlier time points, thus facilitating termination of procedures before the disease reaches a severe stage.

PROJECT 157

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Neural mechanisms of appetitive learning
Key Words	appetitive learning, addiction, associative learning, motivation, neuronal mechanisms
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Exposure to information or ‘signals’ (e.g. sound of ice cream truck) that are linked to palatable foods (e.g. ice cream) shape our urge and motivation to eat. For example, when we see a fast-food sign, we might be reminded about snacks and experience food cravings. We do not fully understand how brain cells actually store and retrieve these types of linked associations. Without understanding this, development of therapies to treat conditions such as excessive food cravings and overeating would be difficult. Using rodents, the aim of this project is to determine how a tiny minority of brain cells called ‘neuronal ensembles’ stores and retrieves memories about food. We will study the cells in brain regions that are important for motivation to satisfy our basic needs (e.g. drinking and eating). We will also study at a molecular and cellular level, what is special about the brain cells that enables to memories to be formed and be retained. This research can be applied to huamans and is important not just for better understanding food-related memory, but also conditions such as overeating and obesity, which are a major burden to health, society and the economy.

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

This project has many benefits largely at the basic science level because when this project is completed, we would have identified the contents of brain cells and their cellular environment that help store and retrieve memories about food. For example, we may find out whether these brain cells contain specialised proteins that make them behave differently from other brain cells that do not participate in food memory retrieval and storage. Indirectly, the results from this research may be useful in the distant future (e.g. 10-15 years), as such information may be useful for creating

better medicines for people that suffer from conditions such as excessive appetite, overeating, and obesity. These types of conditions are suffered by millions of people in the UK and are associated with diseases such as diabetes. We hope that these medicines will specifically target brain cells that control conditions such as excessive appetite, without affecting the brain cells that control normal mental functions such as remembering to buy groceries. Since we are studying learned associations, this research will also provide clues on normal and abnormal mental functioning such as how the brain links and remembers other types of important information that shape our behaviours (e.g. the smell of smoke signaling fire). Such clues may also reveal more about conditions that affect our health such as drug abuse, since the development of drug addiction involves linking information about drugs and the environment where drugs are used (e.g. the sight of smokers creating a cigarette craving).

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

Mice 14,000, Rats 3,500

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

In most of the behavioural tests, the animals are free to move around. These tests may involve a rodent reacting to a tone that predicts food availability (similar to how a pet dog might react before being fed), and/or a rodent performing a response such as a lever press to obtain a food reward. Usually these tests are conducted in an experimental chamber (often called ‘Skinner Box’) that is equipped with levers, lights, speakers, and food dispensers. The animals usually go into this chamber twice a day for 30 minutes at a time, and the training will last from several days to weeks. The animals get used to these chambers very quickly because they may receive rewards in there, and do not exhibit signs of discomfort, akin to a pet rat or mouse going into a new home cage. Where food restriction is required to maintain an animal’s motivation, animals will be monitored for excessive weight loss every day (i.e. we ensure that their weights do not drop by more than 15% compared to animals which have unlimited access to food) and their diet is adjusted accordingly. A minority of animals will have to be slightly restrained during brain imaging in order to clearly see the very fine structures of brain cells. These animals will still be able to move on a ball or flat surface similar to a treadmill. They will be gradually introduced to the restraining and imaging procedures, and wherever possible be given rewards to minimise stress during the imaging process. Surgical work will be carried out under sterile conditions similar to a hospital operation room to reduce the risk of infection. Where possible, gas anaesthetics will be used for rapid recovery, and analgesics or ‘painkillers’ to manage pain. Some animals will be surgically implanted with a thin tube called a ‘guide cannulae’ to deliver substances into the brain to control brain cell activity, or an imaging probe to observe brain cell activity. This will allow us to study

the relationship between brain cell activity and behaviour. This procedure is of moderate severity, but has been shown to be well-tolerated in many peer-reviewed studies. Also, painkillers will be provided during and after the surgery to minimise discomfort. After completing these experiments, all animals are humanely killed using approved euthanasia methods and usually their brain cells will be further analysed using laboratory tools such as a microscope.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We would like to study how food-related memories are stored and retrieved. For these reasons, the use of animals that possess the ability to learn and remember information is essential. It is not possible to investigate such complex processes using cell culture systems, computer modelling, or brain imaging methods in humans. In this project, we will use genetically altered animals because they allow us to answer scientific questions that cannot be answered in normal animals. For example, we can identify the precise brain cells that are involved in learning and memory by using animals which have certain types of brain cells genetically marked, which we can then identify under a microscope. This means we can gain more information, and produce higher quality scientific reports, than if we only studied normal animals. Although mice and rats do not behave completely like humans, their brain circuits thought to control the behaviours of interest here are largely similar (e.g. learning about food). Hence, rodents are a useful experimental tool to study the brain mechanisms of how we store and retrieve food-related memories.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The numbers of animals to be tested will be the minimum number required to obtain reliable experimental results, based on previous experience in the laboratory, and from published studies. Where appropriate, we will use mathematical formulas called ‘power calculations’ to estimate the minimum number of animals we would need to be confident in our results. Also, where possible we test the same animal many times using an approach called ‘within-subject comparisons’ which means we need to test fewer animals overall. Both of these methods allow more reliable experimental results to be obtained and limits the numbers of animals used.

Refinement

Explain the choice of animals 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.

Refinement

This project involves complex behavioural tasks (e.g. learning to make a particular response such as pressing a lever to obtain food) and rodents perform these tasks very accurately. During behavioural training, we carefully observe the animals using a live camera feed, and by examining their training data. These observations are useful for detecting if animals might be unwell. In addition, many aspects of the rodent brain are similar to humans, including which brain areas are important in producing the behaviours that we study. We reduce stress to these animals through use of food rewards in training, rather than punishment. Training using food rewards can be very successful and is widely used to train service animals (e.g. police dogs). To improve their well-being, animals will be group housed as rats and mice are social species which naturally choose to live in groups. Also, they will usually undergo behavioural tests in the same familiar location in a dark and quiet room that minimizes stress. In general, we pay lots of attention to the well-being of the animals (especially after surgeries), including trained and qualified personnel examining a wide variety of behavioural and physical signs. If necessary, animals that are unwell would be humanely euthanised to prevent excessive suffering.

PROJECT 158

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The role of Reactive Oxygen Species (ROS) in neuromuscular homeostasis during ageing and disease
Key Words	Neuromuscular Ageing, Reactive oxygen species
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Age-related loss of skeletal muscle mass and function is a major contributor to frailty in the elderly. This project is designed to:

1. Identify mechanisms underlying loss of muscle mass and function during ageing.
2. Identify the role of neuromuscular degeneration in the loss of muscle mass and function.
3. Identify potential interventions to preserve muscle mass and function during ageing.

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

The research proposed is of high importance and we anticipate that the outcomes of this study will lead to a greater understanding of the mechanisms that underlie the loss of muscle mass and musculoskeletal function in older individuals. Knowledge of those mechanisms will then be used to design logical interventions to attempt to correct the processes involved with potential benefits in reduction of loss of muscle mass and function and prevention of physical frailty in older individuals.

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

The maximum number of animals used in the experimental protocols will be 1500 over 5 years. These will be young, adult or old mice and genetically modified mice.

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

The loss of muscle mass and decline in strength occur inevitably during ageing and these experiments are designed to understand how this process occurs and how it might be prevented. The expected adverse effects, of the procedures, although very rare, may therefore be associated with muscle weakness and result (sporadically) in change in movement. Thus in some experiments a skeletal muscle of anaesthetised mice will be stimulated to contract to understand the effects of exercise on age-related loss of muscle, while in other studies a small nerve may be cut to understand the role played by nerves in maintaining muscle during ageing. In both of these example experiments, mice will show no prolonged signs of disability. Any adverse effects of the experiments will be monitored for through observing the mice daily and weighing the mice. Any significant changes or moderate changes lasting more than 3 days will result in animal cull and tissue collection. At the end of the protocols mice will be culled.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Due to the nature and complexity of the experimental design it is not possible to use human tissue, therefore the proposal requires the study of living mice. In addition, because of the need to study the interplay between nerve and muscle cells and ageing of post-mitotic tissues, it is not feasible to use a cell culture based approach. It is not possible to imitate the innervation of muscle ex vivo. The rodent is the lowest species that demonstrates comparable nerve-muscle interactions. Mice are the lowest vertebrate group possible in this study and the availability of genetically altered mice will provide definitive data necessary to achieve the objectives for this study. Other species have been considered but deemed unsuitable for these experiments, such as zebrafish, drosophila or nematode. Ageing is a whole organism phenomenon. The ability to examine the effect of age on muscle structure and function is not possible in cell culture. The alternative is the use of primary culture to generate muscle from aged mice. However, this raises the problem of the lack of a suitable model to perform muscle force measurements in culture.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The proposal has been designed with the help of statistical advice to minimise the number of mice used. Power calculation performed in support of each procedure will ensure that the minimum number of mice required to achieve statistically significant results will be used.

Refinement

Explain the choice of animals 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.

Refinement

Most of our work will be carried out in mice. Mice are chosen for these experiments due to the similar physiology between mouse and human musculoskeletal system. Mice are the lowest vertebrate group possible in this study and the availability of genetically altered mice will provide definitive data necessary to achieve the objectives for this study. The mouse is relatively short-lived, therefore the effect of age on musculoskeletal structure and function can be fully documented over a relatively short timescale.

A number of check points for minimising animal suffering are included in the protocol design, including clear end points, shortest time necessary and minimal number of interventions. The applicants have considerable experience in all the techniques used.

The general experimental designs and methods of analysis of the results have been discussed[REDACTED]

PROJECT 159

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	The role of Reactive Oxygen Species (ROS) in neuromuscular homeostasis during ageing and disease
Key Words	Neuromuscular Ageing, Reactive oxygen species
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Age-related loss of skeletal muscle mass and function is a major contributor to frailty in the elderly. This project is designed to:

1. Identify mechanisms underlying loss of muscle mass and function during ageing.
2. Identify the role of neuromuscular degeneration in the loss of muscle mass and function.
3. Identify potential interventions to preserve muscle mass and function during ageing.

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

The research proposed is of high importance and we anticipate that the outcomes of this study will lead to a greater understanding of the mechanisms that underlie the loss of muscle mass and musculoskeletal function in older individuals. Knowledge of those mechanisms will then be used to design logical interventions to attempt to correct the processes involved with potential benefits in reduction of loss of muscle mass and function and prevention of physical frailty in older individuals.

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

The maximum number of animals used in the experimental protocols will be 1500 over 5 years. These will be young, adult or old mice and genetically modified mice.

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

The loss of muscle mass and decline in strength occur inevitably during ageing and these experiments are designed to understand how this process occurs and how it might be prevented. The expected adverse effects, of the procedures, although very rare, may therefore be associated with muscle weakness and result (sporadically) in change in movement. Thus in some experiments a skeletal muscle of anaesthetised mice will be stimulated to contract to understand the effects of exercise on age-related loss of muscle, while in other studies a small nerve may be cut to understand the role played by nerves in maintaining muscle during ageing. In both of these example experiments, mice will show no prolonged signs of disability. Any adverse effects of the experiments will be monitored for through observing the mice daily and weighing the mice. Any significant changes or moderate changes lasting more than 3 days will result in animal cull and tissue collection. At the end of the protocols mice will be culled.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Due to the nature and complexity of the experimental design it is not possible to use human tissue, therefore the proposal requires the study of living mice. In addition, because of the need to study the interplay between nerve and muscle cells and ageing of post-mitotic tissues, it is not feasible to use a cell culture based approach. It is not possible to imitate the innervation of muscle ex vivo. The rodent is the lowest species that demonstrates comparable nerve-muscle interactions. Mice are the lowest vertebrate group possible in this study and the availability of genetically altered mice will provide definitive data necessary to achieve the objectives for this study. Other species have been considered but deemed unsuitable for these experiments, such as zebrafish, drosophila or nematode. Ageing is a whole organism phenomenon. The ability to examine the effect of age on muscle structure and function is not possible in cell culture. The alternative is the use of primary culture to generate muscle from aged mice. However, this raises the problem of the lack of a suitable model to perform muscle force measurements in culture.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The proposal has been designed with the help of statistical advice to minimise the number of mice used. Power calculation performed in support of each procedure will ensure that the minimum number of mice required to achieve statistically significant results will be used.

Refinement

Explain the choice of animals 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.

Refinement

Most of our work will be carried out in mice. Mice are chosen for these experiments due to the similar physiology between mouse and human musculoskeletal system. Mice are the lowest vertebrate group possible in this study and the availability of genetically altered mice will provide definitive data necessary to achieve the objectives for this study. The mouse is relatively short-lived, therefore the effect of age on musculoskeletal structure and function can be fully documented over a relatively short timescale.

A number of check points for minimising animal suffering are included in the protocol design, including clear end points, shortest time necessary and minimal number of interventions. The applicants have considerable experience in all the techniques used.

The general experimental designs and methods of analysis of the results have been discussed[REDACTED]

PROJECT 160

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The role of movement in prenatal skeletal development
Key Words	Skeletal development, Fetal movements, Biomechanics, Spine, Joint
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall aim of this project is to understand how mechanical forces due to fetal movements (the movements of a baby in the womb) affect development of the skeleton, and in particular the bones, joints and spine. There are a number of conditions affecting the skeletons of newborn babies that are linked with abnormal fetal movements, such as hip dysplasia (where the hip joint is unstable), arthrogryposis (where multiple joints are malformed and bent) and congenital scoliosis (abnormal spinal curvature and/or vertebral shape). Hip dysplasia and congenital scoliosis occur in about 1 in 1,000 births and arthrogryposis occurs in 1 in 3,000 births. However, the role of fetal movements in skeletal development is poorly understood, for example, when is the most critical time for movement, and can we artificially increase movement to reduce the effects on the skeleton.

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 key benefits from this project are that it will: a) Give us a better understanding of skeletal development. This is relevant to tissue engineering of bone and cartilage. b) Provide insight into developmental conditions affecting the skeletal system in newborn babies, such as hip dysplasia, congenital scoliosis and arthrogryposis. In particular we hope to be able to offer insight to patients and their parents on why they might have developed the condition c) Identify possible means of reducing the effects of abnormal fetal movements on skeletal development by investigating the effects of applying massage, or introducing an exercise regime, in pregnant mice of embryos without movement. This could lead in the future to preventative treatments for conditions affecting human babies, such as hip dysplasia.

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

Only mice will be used. We use genetically modified lines in which the fetal movements of homozygous (having an identical pair of the gene of interest) embryos are absent or abnormal. I expect that 1,500 or less genetically modified animals will be humanely killed over the course of the 5 year term.

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?

As heterozygous (having a dissimilar pair of the gene of interest) animals have no phenotype, and all homozygous animals will be harvested prior to being born, the vast majority of animals are expected to suffer no adverse effects, with the severity being sub-threshold. At the end, all animals, will be killed using humane methods.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

[REDACTED]

However, the chick has no intervertebral disc (a key structure of the human spine) and so any studies of this disc must be conducted in a mammal, of which the mouse is the most suitable. Furthermore, in order to be able to fully understand the importance of fetal movements for human diseases, we also need to study an animal that develops in a womb, as the environment of the egg is very different to that of the uterus.

Joint shape data gathered under this license will contribute to a computer simulation of joint growth and shape change, which will help us understand how mechanical forces direct and affect joint development. . This stage of the project will use data from the previous three stages, and will therefore not necessitate any further animal use.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We save all skeletal tissues from the embryos, so each embryo is used for at least two different studies (joint and spine). We use inbred strains to minimise variation. We do 3D imaging so that shape is easily characterised, reducing the number of

animals needed. Experiments will be planned so they can be published in accordance with the NC3Rs' ARRIVE guidelines.

Refinement

Explain the choice of animals 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.

Refinement

Bearing in mind that we need to use a mammal for studying the disc, and the mechanical environment of the uterus), the mouse is the most refined animal model for fulfilling the objectives of the research.

Our breeding protocols are designed so that embryos with a harmful mutation are harvested prior to being born.

Procedures other than breeding will cause minimum harm to the animals. The biggest welfare cost for any individual animal will be two injections, with general anaesthetic up to a maximum of four times, which will only apply to a small subset of the animals

Animals exhibiting any unexpected harmful characteristics will be killed using a humane method or in the case of individual animals of particular scientific interest, advice will be sought promptly from the local Home Office Inspector. Any animal showing signs of suffering that are greater than minor and transient or in any way compromise normal behaviour will be immediately killed using a humane method.

PROJECT 161

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Gene function in cardiovascular disease
Key Words	Cardiovascular, Heart failure, Genes, Mouse
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project will identify genes involved in cardiovascular disease and will determine their role in both the healthy and diseased cardiovascular system. In particular, there is a need to understand the genes involved in the development heart failure because current treatments are not effective.

Where our knowledge of the fundamental role of particular genes in the cardiovascular system is more advanced we will provide new insights in developing treatment options for heart failure by testing whether drugs which affect the action of these genes are able to halt/reduce the development of heart failure and its associated diseases. We will also determine whether treatment with genetically modified stem cells can improve cardiac function following a myocardial infarction.

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)?

Since we are directly studying the relevance of these genes in established animal models which mimic the most prevalent human cardiovascular diseases it is anticipated that our studies will provide novel information regarding such genes and reveal potential new targets for the treatment of heart failure and associated diseases such as high blood pressure, arrhythmias, and heart attacks. The ultimate aim is to develop more effective treatments for heart failure and its associated diseases such as high blood pressure, arrhythmias, and heart attacks. By characterising the role of genes in the healthy and diseased cardiovascular system this project will provide an essential link between basic scientific research and future medical treatment because once we know the identity of the proteins and pathways responsible it may be possible to develop treatment options to either reverse/enhance their detrimental/beneficial effects.

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

During this 5 year project we expect to breed approximately 12,500 mice. The majority of these mice will have a genetic modification which will enable us to study the role of our genes of interest in the cardiovascular system. We expect that of these mice 4,150 will be used in regulated procedures, tissue/blood will be collected from an additional 3,500 mice. Mice are used in this project since these remain the most suitable animals for genetic modification experiments. As the main focus of the described work is to study gene function in the cardiovascular system mice have been chosen because the anatomy and physiology of the heart and vasculature is both well documented and physiologically similar to humans.

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?

To investigate the role of a gene in cardiovascular health and disease we will breed mice carrying mutations in the genes we are studying. Genetically normal mice will be bred to act as control animals for the experiments. To determine the effect of the gene on cardiovascular performance mice will undergo a series of tests which mimic those performed by a cardiologist on human patients eg heart rate and rhythm will be measured by ECG, blood pressure recorded, cardiac ultrasound used to assess the structure and pumping of the heart. This series of tests has a mild severity as there is no significant impact on the well-being and general condition of the animal. At the end of the experiment the animal will be killed and blood, urine and tissue will be collected for biochemical analysis. To determine the effect of the genes in the diseased cardiovascular system we will generate models to mimic human cardiovascular disease. Cardiac hypertrophy, hypertension, and myocardial infarction, all of which potentially lead to heart failure, will be modelled and the effect on cardiovascular function will be assessed using the series of tests described in the paragraph above. Both hypertrophy (cardiac growth) and hypertension can both be induced by surgical implantation of a small device to release drugs to raise blood pressure or lead to hypertrophy. The surgical procedure to implant the device is carried out under general anaesthesia with appropriate analgesia to relieve any post-surgical pain. Animals fully recover from the surgery within 24-48 hours and the hypertrophy/hypertension develops over the following 7-14 days. The animals do not display symptoms of the hypertrophy/hypertension unless it develops into heart failure. The visible symptoms of heart failure are lethargy, lack of interest in food, drink and surroundings and laboured breathing. Mice exhibiting these symptoms will be humanely killed. A surgical procedure to constrict the aorta will also result in hypertrophy as the heart works harder to pump the blood around the body. Again this procedure is carried out under general anaesthesia with analgesia as described above, with the hypertrophy developing over 1-5 weeks. Myocardial infarction (MI), a heart attack will be induced under general anaesthesia with appropriate analgesia by

constricting one of the major blood vessels of the heart. As in the human population a number of mice (~10%) will die of acute heart failure within 24 hours of the MI and a further ~13% will die of cardiac rupture 4-5 days after the MI. These deaths are very sudden and are instantaneous. Over the following 1-5 weeks the heart forms a scar where the MI has occurred and cardiac hypertrophy and subsequent heart failure may occur.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

As the main focus of the described work is to study gene function in the cardiovascular system mice have been chosen because the anatomy and physiology of the heart and vasculature is both well documented and physiologically similar to humans . Insights into the underlying mechanisms of heart development and function can be gained from studying lower organisms such as *drosophila*, fish and worms but they do not possess the four chambered heart and subsequently have a different circulatory system.

There has been a recent increase in the use of stem cells as an experimental model. There is a lack of human cardiovascular tissue available for research which has led to the development of techniques to induce cardiomyocytes from human stem cells. Our own work involves transforming human skin fibroblasts to stem cells (known as induced pluripotent stem cells-iPSC) and from there growing the cells under such conditions to convert them to cardiomyocytes (iPS-cardiomyocytes). These cells have many of the characteristics of human cardiomyocytes and thus we are developing them as a model system in which to characterise the effect of gene modification on hypoxia and hypertrophy and to understand the signalling pathways involved.

This *in vitro* system can complement and enhance our research involving animals but will not act as a replacement for experiments which require the understanding of gene function within the context of the whole organ. These factors can only be investigated *in vivo* within the context of the whole organ because the cardiovascular system responds to factors carried in the blood and from cell to cell, as well as to the physical forces imposed by the beating heart and blood pressure. In combination with gene modification these factors will affect the disease process.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The personnel involved in this project have extensive experience of all the key techniques to be used within this project; including small animal surgery, cardiovascular phenotyping and induction of cardiovascular dysfunction. Their experience has and will continue to ensure that procedures are carried out efficiently, thereby minimising the suffering to animals, and the numbers required for reproducible results.

Genetically modified and wild-type litter-mates are analysed in groups of the same age and sex to eliminate variation in the results due to anything other than the genetic modification. This regimen of using animals of the same age, sex and strain is essential, since such differences affect heart rate, blood pressure and indices of contractility.

The series of tests to analyse cardiovascular function under basal and stimulated conditions was designed and refined in order to obtain information on several factors from each animal, whilst not raising the severity level beyond mild, as the procedures have no significant impact on the well-being and general condition of the animal. This includes collection of cardiovascular tissue and blood that will be used extensively in biochemical, molecular and *in vitro* studies.

The use of sham operated animals versus non-treated controls will be considered for each experiment. Where possible control data from previous experiments will be used, negating the use of further sham controls. Where available data comparing sham and untreated controls will be compared and where possible untreated controls have been used. Where it is considered scientifically valuable to use sham-operated controls we have found that the number of animals in the sham group can be lower than the number of treatment animals as the variation and therefore standard deviation between mice is lower.

Refinement

Explain the choice of animals 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.

Refinement

The work carried out in this project studies the function of a number of genes implicated in cardiovascular disease, particularly heart failure and its co-morbidities including hypertrophy, hypertension, and myocardial infarction. It is essential to understand the role of these genes in normal cell function and to study the consequences of abnormal function. We are directly studying the relevance of these genes in established animal models which mimic the most prevalent human cardiovascular diseases.

To minimise the harm to the animals used in these studies we have selected and refined approaches and techniques which cause the least pain, distress and lasting harm whilst being able to meet our objectives.

The effect of any genetic modification on the health of the animal is unpredictable, as is the effect of a procedure on a genetically modified animal, so all animals are inspected at least once a day. Any animals showing signs of distress are evaluated in consultation with the veterinary surgeon and humanely killed if the distress cannot be averted. The majority of genetically altered strains we are currently studying do not have observable phenotypes that impact on behaviour. One strain results in homozygous embryonic lethality at day 4 of development – but breeding is now maintained by crossing wild type mice with those carrying a heterozygous mutation.

All surgical techniques are carried out under general anaesthetic to ensure that the animal does not feel any pain, and any post-surgical pain is treated with the use of analgesics. Any animal in which pain is uncontrolled, or which has significant surgical complications, or whose general health deteriorates, is humanely killed.

Physiological analyses (echo, haemodynamics) are also performed under general anaesthetic, in the majority of cases the animal is under terminal anaesthetic from which it does not recover. ECG may also be performed on conscious animals in a non-regulated procedure; this is a quick procedure which is not stressful and does not require anaesthesia.

Any stress caused by administration of pharmacological agents is momentary as the injection is given. Where applicable mini-osmotic pumps are used to administer hypertrophic, hypertensive and other pharmacological agents. Although their use initially involves minor surgery, the technique in our experience leads to highly reproducible and consistent results requiring fewer animals per experimental group. The use of osmotic pumps also reduces handling and stress in animals.

Coronary artery ligation for induction of myocardial infarction is a severe protocol. This can lead to death if the mice develop acute heart failure or if the heart ruptures but these are minimized by careful placement of ligatures, refined surgical techniques and post-operative care.

PROJECT 162

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Cellular mechanisms of organ fibrosis and repair.
Key Words	Fibrosis, Scarring, Tissue repair, Pericytes, Myofibroblasts
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
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No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Although organ fibrosis represents a massive healthcare burden worldwide, currently available treatments are severely limited and organ transplantation is, in many cases, the only effective therapy for end-stage fibrotic disease. However, limited donor organ availability, high cost and co-existing illnesses in potential transplant recipients mean that, on a global scale, organ transplantation can only be offered to a small percentage of patients suffering from the complications of fibrosis. The development of effective anti-fibrotic therapies is therefore essential to improving patient care. Myofibroblasts are specialised cells within our organs that are the major source of scar tissue during organ scarring (fibrosis). Therefore these cells are an attractive target to study further in our search for effective new treatments for organ fibrosis. In order to study the molecules in myofibroblasts that might be responsible for causing scar formation during tissue fibrosis, we will use genetically modified mice that allow us to remove specific molecules within myofibroblasts to see if this reduces scar formation in mouse models of organ fibrosis.

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

1. A greater understanding of the cell biology of organ scarring and repair, which will allow us to develop better, more potent treatments for animals and human patients with organ fibrosis. 2. The development of effective small molecule or antibody based treatments that limit organ injury, accelerate tissue regeneration and limit fibrosis development. 3. The establishment of and increasing experience with mouse models of liver, kidney, lung and cardiac injury and fibrosis which may allow other researchers throughout the wider research community to study the cell/molecular biology of tissue injury and regeneration. 4. The effects on organ transplantation are

potentially far reaching. Effective therapies for acute liver injury, as seen in paracetamol poisoning, might avoid the need for liver transplantation altogether. This would expand the potential pool of donated livers for patients with chronic liver disease and cancer. Additionally, developing treatments to retard the progression of liver, kidney, lung or cardiac fibrosis once injury has taken place could reduce the need for transplantation (and/or dialysis in the case of chronic kidney disease) and therefore reduce the burden of morbidity and mortality associated with these therapies. 5. Identification of blood test or urine test based biomarkers of fibrotic disease and response to therapy in fibrosis of the liver, kidney, lungs or heart.

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

We expect to use approximately 13,500 mice over 5 years.

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

We plan to use different mouse models to induce fibrosis in different organs including the liver, kidney, lung and heart. As detailed above, these models are predominantly of moderate severity and therefore we expect minimal adverse effects for the animals using these models. The animals will be culled humanely at the end of the experiment

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Our group and others within our Centre have many years of experience working with mouse models of organ fibrosis. We will use data from our previous work and in-depth statistical analysis to ensure that we use the minimum number of animals possible. Furthermore, we have developed an imaging technique that allows us to sequentially image organ fibrosis longitudinally in the same mouse. This means we can follow the course of organ fibrosis and repair in a single mouse, giving us a very large amount of information, and greatly reducing the number of animals we will use.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Our group and others within our Centre have many years of experience working with mouse models of organ fibrosis. We will use data from our previous work and in-depth statistical analysis to ensure that we use the minimum number of animals possible. Furthermore, we have developed an imaging technique that allows us to sequentially image organ fibrosis longitudinally in the same mouse. This means we can follow the course of organ fibrosis and repair in a single mouse, giving us a very large amount of information, and greatly reducing the number of animals we will use.

Refinement

Explain the choice of animals 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.

Refinement

The mouse models to be used have been extensively evaluated in the literature, and we have extensive experience of these clinically relevant models and parallel studies in humans will ensure refinement to best model the human conditions under study. The earliest study endpoints are utilised in the experiments. Furthermore, over the past few years we have further refined some of our organ fibrosis models to reduce the severity of the model. For example, we now routinely house mice at 28 ^{o}C following bile duct ligation surgery which has reduced mortality rates with this model to virtually zero.

PROJECT 163

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Novel immunotherapeutic strategies to treat cancer
Key Words	Immunotherapy, Cancer, Antibody, Immunology
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
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No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Cancer affects 1 in 3 people in the UK during their lifetime and is the cause of over 40% of premature deaths. Treatment frequently involves surgery, usually accompanied by chemotherapy or radiotherapy. However, patients often relapse due to the survival of small numbers of tumour cells and despite decades of work on treatment regimens, the survival for many cancers remained unchanged until very recently. Clearly there is a need to develop new therapies for use either as an alternative to or in combination with conventional treatments. Utilising the patient's own immune system to seek and destroy remaining cancer cells (termed immunotherapy) is an attractive adjunct to current treatments as this can potentially be performed with maximal specificity and minimal toxicity. After many years of disappointing results recent success in cancer immunotherapy has reinvigorated the hypothesis that the immune system can control many if not most cancers, in some cases producing durable responses in a way not seen with many small molecule drugs

The overall aim of this project is to explore the utility of new anti-cancer reagents for use in the clinic. The specific objectives are:

1. To produce and characterize new anti-cancer reagents.
2. To determine the therapeutic effects of anti-cancer reagents, the mechanism of these effects, and how they may be improved.
3. To develop strategies to promote/modulate a patient's own immune responses to cancer and to understand the underlying immune mechanisms.
4. To understand the way in which tumours develop (tumorigenesis) with the aim of developing reagents that can inhibit the 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)?

Immunotherapy has the potential to provide long-lasting protection from tumour relapse in patients. The principles obtained from our work will also inform the fields of clinical infection, autoimmunity, transplantation and allergy as well as veterinary science.Animals will be used in experiments beteween

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

Animals will be used in experiments between May 2017 and April 2022. On the basis of our current research, it is estimated that we will use approximately 33,000 animals during this 5 year period. We will apply for amendment to the licence if monitoring shows that this is likely to change significantly.

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

The vast majority of the experiments will result in no adverse effects. When using tumour models, mice will be culled at the humane endpoint. In some cases the administration of immunomodulatory substances may cause transient adverse effects, but these will remain within the moderate severity limit, otherwise the mice will be culled.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We are committed to replacing mice where possible and we evaluate immunotherapeutic agents on cell lines in vitro when we can. However, immune modulating agents act upon multiple cell types across the body concurrently and this cannot be adequately modelled in vitro at the current time. Similarly, to study the interactions between an ongoing immune response and a growing tumour, or to evaluate immune-mediated pathology there is unfortunately no viable alternative to in vivo modelling using animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Mice used across experiments are inbred thereby minimising intra-group variability and allowing reduced mouse numbers for experiments. Experiments are always designed with the fewest animals consistent with obtaining statistically valid results.

We have performed Power analysis to determine the numbers of mice required to deliver statistically significant results, although through experience we find we can often use smaller numbers of animals without sacrificing statistical significance. Where appropriate, small pilot experiments are carried out where simple factors such as dose or route of administration are not clear. Where multiple inter-relating parameters are to be evaluated, larger factorial experiments are performed to prevent use of excess mice as controls. In recent years significant technological advances have enabled more information to be obtained from one individual mouse than was previously possible (e.g. using multi-parameter flow cytometry and micro-array technology), enabling multiple parameters to be assessed simultaneously from small samples. These technologies thereby facilitate longitudinal studies and reduce the need to cull multiple mice at different time points to sample from the spleen for instance; we aim to fully exploit these new techniques fully where possible. Tumour cells will be stored frozen when possible to prevent mice being used to passage tumour in vivo.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the least sentient mammal species with an immune system similar to humans. Mice represent a relevant animal model for these studies and the clinical successes now being reported using immunomodulatory drugs against cancer were dependent on data arising from such murine studies. Numerous mouse cancers have been studied and the availability of genetically altered strains, and commercially available reagents aids this research. Environmental enrichment, good husbandry and frequent monitoring ensure high welfare standards. Few adverse effects are anticipated but, should any occur, rapid steps will be taken to ameliorate them or humanely cull affected animals.

Death is not an acceptable end-point for cancer models: we have established end-points for humane culling before pain/distress occurs, based on accepted guidelines. Many tumour lines develop as subcutaneous nodules, allowing easy monitoring of tumour size. However, the visible or palpable size of the tumour is only one of the criteria used for determination of humane endpoint. Experiments will therefore be terminated before tumour size limits behaviours (feeding, drinking, movement) or before or at the first signs of, tumour associated symptoms or poor condition of the animal according to well defined guidelines (e.g. facial expression scales; www.nc3rs.org.uk/assessment-pain-using-facial-expressions-laboratory-mice-rats-rabbits-and-macaques). Occasionally, following therapy a subcutaneous tumour resolves from the inside out giving the appearance of ulceration; we have

adopted a scoring system from Lloyd and Wolfensohn in the Handbook or Laboratory Animal Welfare and Management to ensure that these are managed with minimum adverse effects to the mice. While the maximum severity limit for much of the work to be conducted under this PPL is set as ‘moderate’, through experience and good management of the mice, we have found under our existing PPL that the actual severity of most experiments is ‘mild’.

PROJECT 164

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Targeting IL-17 driven pathology
Key Words	IL-17, ROR gamma t, Small molecule inhibitors, Innate Lymphoid Cells, Inflammation
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aim of this project is to determine whether a molecular pathway known to have a key role in inflammation (IL-17 pathway) can be effectively targeted and reduced through the use of small molecule inhibitors. Our initial experiments will use mouse models which genetically delete molecules in the pathway, providing a very robust system to understand exactly what these molecules do and what will potentially happen when targeted with inhibitors. These studies will then inform experiments where small molecule inhibitors targeting these transcription factors are assessed trying to recapitulate results from the genetic targeting. These small molecule inhibitors will be selected as those already being proposed for therapeutic use and at the pre-clinical stage of development, using existing data on efficacy and toxicology.

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

The work proposed here will generate fundamental knowledge on the molecules that control IL-17 responses. IL-17 responses are key drivers of inflammation and targeting this pathway has substantial therapeutic potential particularly for inflammatory conditions such as inflammatory bowel disease. New recent data from our lab indicates that targeting specific pathways offers new hope for controlling inflammation through blocking damaging IL-17 responses produced by T cells, whilst leaving protective responses from other cells intact. We have established genetically modified mice that enable us to test this hypothesis and these will define the requirements of the two main IL-17-producing cell types for certain molecules in controlling the IL-17 response. Using a series of mouse infection models we will determine the requirements of the different cells. Informed by these experiments, we will then use small molecule inhibitors already at the pre-clinical stage of

development (through industrial collaborations) to test the ability of such inhibitors to block the IL-17 responses. These experiments will provide key data demonstrating the efficacy of the pre-clinical reagents. Importantly, the IL-17 models developed will provide crucial mechanistic data into their effects on cells producing IL-17. We aim to demonstrate how T cell driven production of IL-17 can be blocked to limit inflammation. We will identify the consequence of this inhibition on the T cell response. The benefit of this work will be the translation of our initial basic studies showing that targeting transcription factors in the IL-17 pathway can block inflammation and thus offer therapeutic benefit in the clinic. The knowledge gained here will reveal the effects of specific small molecule inhibitors on immune cells in vivo. This data is needed to enable the subsequent testing of these molecules in patients and the generation of new therapeutic approaches in the clinic.

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

Approximately 6,500 mice will be required to perform the planned experiments over the five year time period.

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?

In the course of these experiments, animals will necessarily be subjected to injections, oral dosing, blood sampling and/or modification of their diet. In many of our experimental models the mice will only experience a transient discomfort from an injection in for example the tail vein or by oral dosing for compounds given through the mouth. We have established model infections where the response is induced by bacteria normally found living in the tissue without causing clinical signs. In these experiments, the mice will suffer only the discomfort of introducing the bacteria to this site, for example inoculation into the nose. In some experiments the mice will suffer more significant effects such as local skin inflammation, typically on the ears, which will become slightly inflamed and thickened. In our infection studies the animals will likely suffer discomfort associated with the site of infection, for example inflammation in the mouth due to oral candida infection. Where intestinal infections are used, mice will suffer intestinal discomfort due to local inflammation here which may result in diarrhoea. In all cases adverse effects will be minimised by the use of the most refined techniques by skilled staff, and humane endpoints have been predefined. Infection studies will be conducted for the minimal period of time to address the scientific question and the minimal dose to elicit the desired response. In systemic responses mice are likely to experience fever-like symptoms. Mice are expected to show some weight loss. Careful observation of animals throughout the infection and the use of scoring systems will ensure humane end points are identified and used as appropriate. All mice will be killed humanely at the end of the protocol or should clinical end points be reached, then prior to the end of the protocol

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

This project requires animals as immune responses must be analysed within live animals rather than in a test tube to accurately model the complex dynamics of what actually happens in the body. This work is required to provide clear basic information on the ability of potential therapeutic agents to work and as such it is the first step in translating this scientific work. There are not reliable non-animal alternatives for modelling how these compounds would affect cellular responses and the use of such compounds in patients absolutely requires testing in animal models to really assess how they work and to try to rule out possible adverse effects.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have developed sophisticated models of responses where a high degree of reproducibility between mice reduces mouse to mouse variation enabling smaller group sizes. We will only use compounds for which established toxicity data exists sparing the need for screening experiments that use large numbers of mice. Small scale pilot experiments will quickly identify dosing for infectious agents using the minimal number of mice required to do this. Experiments will be designed following ARRIVE guidelines and using power calculations and [REDACTED] or the local NC3Rs advisor. We constantly re-evaluate our experiments in light of new data in order to ensure optimal design and minimal animal useage to ensure robust scientific data

Refinement

Explain the choice of animals 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.

Refinement

Mice are an excellent model for the human immune system and have been extensively characterised and validated. These animals provide the best means for analysis given the wealth of reagents available and the wide range of genetically modified mice that enable precise mechanisms to be tested, facilitating the development of therapies for human use. Many of the methods described are

established in the lab and all have been selected as models providing robust data without causing overt clinical signs where possible.

General approach:

- In general our approach has been to ensure responses can be measured without overt disease, thus minimising adverse effects to the mice and every effort has been made to develop refined techniques causing minimal clinical side effects.
- All infection studies will be established through initial pilot experiments identifying the minimal dose and duration required.
- The infection models we have chosen are those that enable precise assessment of the IL-17 response, whilst minimising adverse effects.
- We have and will continue to collaborate with others researchers with experience in using some of the animal models we use to avoid unnecessary animal use and reducing adverse effect to the minimum possible.

Specific examples of refinements include:

- We analyse responses of host cells to bacteria that naturally colonise the intestine. We now understand that these responses which do not show any clinical signs, are informative of other responses.
- We use studies of immune cell populations naturally occurring within the mice rather than transferring certain cells into our mice at the beginning of the experiment. This reduces the experimental procedures experienced by the mice and also enhances the quality of the resulting data since our experiments are more physiological. Rather than provide tamoxifen (a drug used in some of the mouse models to induce gene expression) by repeated injection, food containing tamoxifen is used. Where this is not possible, oral administration rather than via an injection into the peritoneal cavity will be used.
- Only small molecule inhibitors for which clear toxicity data exists will be used, acquired through industrial collaboration. This will focus our studies to proven compounds with significant therapeutic potential. We will not screen unknown compounds which may have adverse effects.

PROJECT 165

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Targeting inflammation in cardiovascular disease
Key Words	Heart, Blood vessels, Cardiovascular disease, Inflammation
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Our research interests focus on the regulation of the cardiovascular system in health and disease in an attempt to provide a better understanding of the mechanisms involved, and for the design of novel therapeutics. In particular, we are interested in the role of the endothelium, which lines the inside of blood vessels, in controlling cardiovascular function. Many substances produced by the endothelium, including nitric oxide (NO), prostaglandins and the kinins, are all important in the control of the cardiovascular system and manipulation of these naturally occurring substances form the basis of drug treatment for heart 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)?

Since inflammation is believed to be an important step in the pathogenesis of a number of cardiovascular disease including heart attacks, high blood pressure and shock a better understanding of the actions of these substances might explain some of the abnormalities of blood vessels that are seen in these disease, and suggest new treatments.

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

All studies will be conducted in rodents, the vast majority in mice to exploit transgenic ('knockout') technology. This integrated programme of work will run for 5 years and will utilise approximately 1000 rats & mice per year.

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

The protocols described in this licence allow us to model various aspects of inflammation in the cardiovascular system and to investigate the efficacy of newly developed compounds in the inhibition of this inflammation. Animals will undergo exposure to procedures that cause cardiovascular disease in humans. For example, some will have their coronary arteries blocked to cause a heart attack or heart failure; others will be exposed to an environment mimicking high altitude which causes pulmonary hypertension; some techniques involve administering inflammatory substances to local sites (e.g. the foot) to evaluate how this might be modified by drug treatment. In all cases, protocols are designed to cause the least discomfort (e.g. by the use of anaesthesia and analgesics) and interventions used are always the minimum consistent with the scientific objective. At the end of all studies, animals will be euthanised humanely.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We plan to employ a number of cell-based techniques prior to and during studies involving regulated procedures to guide and minimise animal usage in addition to replacement wherever possible (including the use of human tissue). However, whilst this reductionist approach can aid in the understanding of new biological mechanisms, and thereby the development of new classes of drugs for the treatment of cardiovascular disease, because of the dynamic interactions that typify the course of the disease, to facilitate the identification and subsequent development of therapeutic agents it is also necessary to use animal models. Whilst there are few animal models that faithfully reproduce all the pathology of the analogous human condition, it is possible to identify fundamental processes that may either reveal new targets or act as screens for drug testing. Thus, provided one is aware of the limitations of animal models they play a valuable role in the development of novel and more efficacious medicines.

This project licence includes the breeding of animals in which nitric oxide, prostaglandins and kinins (and other related substances) have been ‘knocked-out’ (i.e. artificial deletion of the genes) for use in our research. This is necessary since selective inhibitors of these substances either have yet to be identified or have proved inconclusive in studies of whole animals and humans. Whilst cell culture has helped enormously in our basic understanding of the biochemical processes involved in the production of these substances, the impact upon disease of altering the production of these natural substances can only be assessed in the whole animal. This application seeks to gain permission to use these ‘knockout’ breeding colonies. Mice are used for these studies since the technology of genetic manipulation has

been advanced in this species particularly and indeed all of the genes that we are particularly interested in have been ‘knocked out’ in mice only. The use of such ‘knockout’ mice will enable us to determine the roles and relative importance of the above substances in physiology and in various cardiovascular disorders.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will utilise cells and tissues initially and concomitantly with animal models to guide and optimise the latter thereby minimising animal usage. Transgenic ('knockout') mouse colonies will be used extensively in this programme of work (since specific pharmacological tools are lacking) and we will use the minimum numbers of breeding pairs to provide offspring to enable efficient *in vitro* and *in vivo* experimentation. We also have considerable experience and expertise in the experimental approaches outlined in this application, and therefore a huge bank of historical data upon which to draw to determine with good accuracy the minimum number of animals studies to conduct to establish a meaningful effect. For example, in the pulmonary hypertension models the average SD for RVSP is 5%. Thus, to detect a 5mmHg change in RVSP with a power of 80%, 8 animals per group are needed. For heart failure models, the average SD for HW/BW ratios is ~11%, and therefore to detect a 20% decrease in hypertrophy, 10 animals per group are necessary. Finally, the average SD for MABP as measured by telemetric probes is 5%. Thus, to detect a 5mmHg change in MABP with a power of 80%, 8 animals per group are needed. [REDACTED]

Refinement

Explain the choice of animals 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.

Refinement

All of the models described in this licence are mild to moderate; there are no models that fall into the severecategory. Through many years of experience and utilisation of the animal models described in this application, and through collaboration with other academic experts in the field, we have constantly refined the procedures. Each is well-validated and widely-used for investigation of the mechanisms underpinning cardiovascular disease, and gleans robust & informative with minimal animal suffering; analgesics and anaesthetics are used whenever necessary, and close monitoring of all animals undergoing licensed procedures is undertaken. Any animal deemed to be experiencing unnecessary suffering by the investigator or veterinarian will be culled immediately by a Schedule 1 method. The work will be conducted

exclusively in rodents, with the vast majority in mice to enable exploitation of transgenic ('knockout') technology. Individuals working under the auspices of this licence will regularly attend NC3R or similar meetings to keep abreast of new developments.

PROJECT 166

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Improving ruminant livestock production efficiency, quality and health.
Key Words	Livestock Production, Product Quality, Environmental Protection, Animal Health
Expected duration of the project	5 year(s) 0 months

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

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

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project seeks to examine ways of optimising efficiency of ruminant meat production from predominately forage-based systems and the associated health of the livestock. It will examine methods of enhancing product quality, particularly in terms of healthiness, shelf life, hygienic quality, flavour, at the same time as reducing the impact of these systems on the environment and ensuring farm based strategies improve animal health.

The research will also contribute towards the production of healthier food in terms of a leaner product and meat which will contain lower quantities of saturated fatty acids and increased content of specific beneficial compounds (i.e. n-3 PUFA linolenic acid, minerals e.g. Se) in meat. The role of meat as a vehicle to deliver beneficial fatty acids and micro-nutrients through to food products is very important. However, any interventions in production systems must ensure maximum animal health is assured especially around common farm animal diseases (e.g. BVD). Further, food borne pathogens such as E. coli O157 can be fatal to the weakest members of our society; this proposal will provide fundamental knowledge which will help us identify practical methods to prevent infections in animals and humans.

Methane produced during anaerobic fermentation in the rumen represents an energy loss to the host animal as well as contributing to emissions of greenhouse gases into the environment. On a global scale agriculture and in particular enteric fermentation in ruminants produces between 21 and 25% of the total anthropogenic emissions of methane. Over the last 30 years ionophoric antibiotics such as monensin and related compounds have been the single most successful class of rumen manipulators to reduce ruminal protein breakdown and to decrease methane production in rumen fluid. However, legislation (1831/2003; EC, 2003) was introduced within the

European Union to prohibit the use of growth-promoting antibiotics, including monensin and related compounds, in animal feeds. Given the general level of consumer concern over additive use in feeds it is unlikely that chemical inhibitors of protein degradation and/or methane formation are likely to find favour in the market and thus a number of screening projects in recent years have investigated exploiting natural products or processes as alternative rumen manipulators. The information on the effect of feed additives and dietary strategies will be generated in this project to help to reduce ruminant greenhouse gas emissions.

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)?

Currently animal agriculture is perceived as inefficient and wasteful in terms of land and nutrient use, this programme of work will improve the efficiency of animal production systems and the quality of the final product. The outputs of this project are targeted and relevant at various levels of the food chain from the farmer producer, to the meat processor, the retailer and the consumer. In particular, the improved ability to (1) feed ruminants under high forage input systems or sustainable protein systems (2) predict nutrient supply and (3) to manipulate production response will lead to improvements in agricultural efficiency both in terms of use of natural resources and in farm profitability (4) systems can be developed which will reduce incidence of animal disease and associated food pathogens. This will contribute to a reduced reliance on imported feeds, particularly imported protein sources, which is especially prudent following the recent and on-going problems in our industry. Improved knowledge of the interrelationships between growth of an animal and subsequent effects on eating quality (for example tenderness) will improve the quality of the product, which is of benefit to the producer and the consumer.

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

Over the course of the project it is envisaged that approximately 600 sheep and 450 cattle will be used in growth and efficiency trials, with future potential to look at the growing goat industry with maximum 60 goats used during this project.

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

The majority of the animals will experience little more than standard farming procedures. All procedures will not exceed mild in severity (e.g. blood sampling, nasal swabbing and faecal grab sampling). At the end of the trial animals will either be returned to the farm or sent off for commercial slaughter.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

For some of this work there is a requirement to measure various combinations of feed intake, rumen fermentation parameters, product quality, and mineral (particularly N) partitioning between productive (growth and product) and excretion (urine and faeces) purposes. These data can only be collected from live animals, and for the purposes of measuring parameters in relation to meat production there is a clear need to use cattle and sheep.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We operated standard quality assurance procedures (i.e. the BBSRC/Defra/FSA Joint Code of Practice). Statistical advice is sought (from North Wyke statisticians) on all experimental protocols to ensure that maximum information is obtained from the minimum resource. Experimental design is of utmost importance in ensuring that the results generated are statistical strong. Previous experimental results are very useful to defining the variance of a measured parameter and in turn in helping to determine the number of replications necessary to acquire a defined level of statistical power.

Refinement

Explain the choice of animals 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.

Refinement

For much of the work to be carried out the effects of treatments on feed intake, production, composition, and/or nitrogen and methane excretion in/from specific animal groups (e.g. sheep or cattle) is required. Therefore, these animals are most suitable and refined for use in this work, decisions on number of animals will be driven by the statistical design.

Before selecting specific animals for trial work their temperament will be assessed as noted by farm staff to ensure the most appropriate individuals are selected to minimise distress whilst on trial.

All animals will be daily assessed for health and well-being, as determined by alertness, feed and water intake. Any sign of ill health will be reported to the Veterinary surgeon with the animal being removed from trial if symptoms persist or are greater than mild in severity.

PROJECT 167

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Ligament licence repair
Key Words	ligament
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The work to be carried out under this project licence will be done to improve the experience of patients undergoing ligament surgery. It also aims to reduce costs for the Health Service due to shorter stays in hospital. Currently there is a large unmet need for developing technologies and products to enhance ligament surgeries that reduces pain and suffering to patients and decreasing the financial burden on the healthcare system. These technologies and products will help patients worldwide to regain normal lives in terms of their ability to carry out everyday functions which had been prevented either by degenerative joint disease or by trauma. The economic benefits would include fewer days lost at work, fewer hospital days and reduced care costs.

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 benefit is the evaluation of safety of new types of materials to be used in orthopaedic surgery.

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

This licence propose to use sheep, rabbit and goat models and the number of animals will not exceed 3000.

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

The surgical procedures detailed in this licence will cause mild or moderate post-operative discomfort which will be controlled by analgesics and refinements made

from our previous experience. The maximum level of severity will be moderate. The animals will be killed at the end of the protocols.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In vitro testing cannot fully replicate the *in vivo* loading, physiological and anatomical conditions required to demonstrate safety and efficacy of novel fixation devices, therefore animal studies are necessary in the development of new fixation devices.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Consultation with a biostatistician at the planning stage will be actively used to optimise study design, minimise the number of animals required, and meet the study objectives.

Refinement

Explain the choice of animals 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.

Refinement

A thorough investigation into the most relevant species to use for these proposed animal models has been conducted in the previous project licence for ligament repair.

The majority of protocols utilise sheep as this species has bones of the size that will allow implants suitable for humans to be used. We have a great deal of experience with many of the protocols described in this licence. This experience has led to refinements in surgical technique, analgesic regimes and post-surgical care.

PROJECT 168

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Vaccination for Control of Porcine Bacterial Respiratory Disease.
Key Words	Pig, Pneumonia, Vaccination
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The target species of these pneumonia diseases is the pig. They do not affect other animals. Ultimately, the licencing of vaccines for use in animals requires that we demonstrate the safety and the efficacy (effectiveness) of the vaccines in the species for which they are intended.

Alternatives will be and are used where possible. For testing neutrailizing antibody we can use a test in the laboratory using blood cells. Nevertheless, demonstrating protection against a disease requires the interaction of the immune system with pathogen in the whole animal.

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)?

Effective vaccination against enzootic pneumonia and pleuropneumonia. There is a huge burden of pneumonia on the farm. Research is needed to develop and improve vaccines to control these diseases. Furthermore, replacements are needed to allow reduction in the farm use of antimicrobials in animals - particularly pigs and poultry. Development of a model of dermal oedema in the pig to replace pulomonary oedema. This reflects the primary pathological changes in the pig lung but on the skin where it can be observed (refinement), where it is less distressing (refinement) and where several tests can be performed on a single animal (reduction).

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

Pigs. This project will use approximately 970 pigs over a period of 5 years. This value is likely to far exceed the numbers actually used because successful use

under one protocol may reduce or replace the need for animals to be used under another protocol.

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?

1. Immunization to demonstrate the effect of experimental vaccines the adverse effects anticipated will be minimal. No harmful materials will be injected. 2. Generation of pleuropneumonia will be a moderate severity. The effects will be minimised by careful monitoring of the animals, pain relief where animals are in pain and termination of the animals at a stage where no further benefit is likely to be gained.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The target species of these pneumonia diseases is the pig. They do not affect other animals. Ultimately, the licencing of vaccines for use in animals requires that we demonstrate the safety and the efficacy (effectiveness) of the vaccines in the species for which they are intended.

Alternatives will be and are used where possible. For testing neutralizing antibody we can use a test in the laboratory using blood cells. Nevertheless, demonstrating protection against a disease requires the interaction of the immune system with pathogen in the whole animal.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The dermal oedema test is intended to allow a reduction in numbers because several replicate tests may be conducted on the skin of the same animal rather than needing to use one animal per test.

Only the mimimum number of animals will be used to address the scientific question effectively.

Refinement

Explain the choice of animals 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.

Refinement

The pig is the only animal that suffers from pneumonia due to these two pathogens. Alternative animals have been used, particularly outside the UK. However, the results from these investigations have been misleading and are now considered unacceptable since they do not reflect the host-pathogen relationship of Actinobacillus or Mycoplasma. For example, use of the mouse intraperitoneal inoculation with Actinobacillus as a model of pleuropneumonia uses 10,000 times the number of bacteria required to cause the disease in the pig, which is approximately 1000 times the size of the mouse. The specific and subtle interaction of the pathogen with its host is not reflected in the mouse model.

Mycoplasma hyopneumoniae is also specific in its effects on the pig. The interaction of Mycoplasma with host cells is highly species specific. Use of another animal, were it to show an effect, would only need to be repeated and confirmed in the target species.

The dermal oedema test is intended as a refinement such that only mild skin lesions are generated rather than use of the pneumonia model in which the lungs are infected. The intention is that skin oedema will replace lung oedema. Furthermore, the skin is visible so that the effect of antibody on the bacterial toxin may be directly observed in the live animal rather than in the lungs when it is visible only at post-mortem examination.

A further refinement is the regular use of opiate drugs (particularly buprenorphine) to alleviate pain. This has been seen to cause a marked improvement in welfare of animals with pleuropneumonia and will also be used in animals in the dermal oedema test. Since buprenorphine is a partial agonist, other agents such as morphine or fentanyl may be used and assessed for their effectiveness as a further refinement in this disease.

PROJECT 169

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Endogenous control of inflammatory resolution
Key Words	Inflammation, Resolution
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The current treatment of diseases driven by on-going/chronic inflammation such as rheumatoid arthritis, asthma and chronic obstructive pulmonary disease is only effective at dampening the symptomatics of these disease; importantly, they do not afford a cure. In fact, in some cases, current medication may actually unwittingly prolong the disease process. Therefore, there is a great need for the development of new treatments with fewer side effects; greater efficacy and that cure the underlying disease by pushing the disease down a resolution pathway rather than simply alleviating symptoms. Over the next five years I wish to investigate the processes that occur within the body that limit the severity, duration and spread of inflammatory responses. Based on on-going work in my laboratory this may also mean tackling the underlying disease process. This area of inflammation research is called “resolution biology”, where the objective is to understand how the body naturally turns inflammation off, identify the internal switches that help this process work efficiently and ultimately develop drugs based on their model of action. Our overall philosophy is to drive on-going inflammation down a pro-resolution pathway. Finally, we reason that one of the contributing factors to the aetiology of at least some chronic inflammatory disease is that process inherent to driving resolution are dysregulated

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)?

One of the many benefits of the outcome of this research project will be to shed new light on hitherto undiscovered pathways that drive resolution and prevent chronic inflammation and the development of autoimmune diseases. Following from this is the more long-term aim of developing drugs based on the mode of action of these internal pro-resolution pathways. Specifically, develop stable chemical agents based on the structure of soluble pro-resolution mediators and/or developing novel drugs for pro-resolution receptors. The benefit of this aim is that such drugs would drive on-

going inflammation down a pro-resolution pathway and help switch off the underlying disease process Finally, we have developed a number of human models of acute inflammation and resolution, which we are characterizing in great detail. The idea is to (A) introduce complementarity with what we are doing with animals, (B) develop a human platform for testing new anti-inflammatory/pro-resolution drugs and (C) compare and contrast the similarities and differences in the immune responses of animals and humans. This will be particularly helpful to our groups and the wider academic community when deciding on whether research is better off been done in humans rather than rodents. In the process, this will legitimise the greater use of human in translational medicine.

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

We plan to use primarily mice (about 9,000) over the next five years Rats = 2,150

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?

I am personally very keen not to use models that cause local pain, systemic inflammation or any form of distress. Personally, I am very much against this. Its for this reason that we utilise stimuli that trigger transient, asymptomatic inflammation which along with sophisticated platforms for data generation can yield a lot of information about the immune response and its role in the development of chronic inflammation and autoimmunity. There are very few occasions when animals are expected to get temporarily unwell. When these experiments are taking place a researcher experienced in that model will very closely monitor them. Should it seem as though there is a departure (exacerbation) from the expected normal course of the inflammatory response in that procedure, advice will be sought from the NACWO and/or veterinary surgeon.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Inflammation, in response to infection or injury, is a particularly complex sequence of events. It is probably best described, from our perspective at least, as a temporal change in cells trafficking into and out of sites of tissue injury, coupled with concomitant cell death and proliferation alongside physical interactions between these leukocytes, all progressing under the added influence of extrinsic soluble mediators and stromal/parenchymal tissue components. Thus, it’s the very nature of this highly evolved dynamic continuum that can never be adequately replicated in

culture dishes using single cell suspensions. For this reason, rodent species have formed the backbone of much of the research into inflammation and inflammatory pathologies. They are also the first choice for the initial screening of compounds, which are purported to possess the ability to modulate these pathologies. Certainly, once the major cellular players have been identified in vivo and their characteristics either in isolation or in interaction with other cells is elucidated, then in vitro systems will be used. However, the limitation of these in vitro assays in context of the bigger, more biologically relevant and complex data derived from animals and man must be appreciated and importantly, no adequate in vitro replacement for in vivo modelling is available.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We are spending a great deal of time, money and effort in establishing and characterising human models of acute inflammation. These include the injection of killed bacteria and vaccines into healthy volunteers (to understand basic processes of inflammation/resolution) as well as patients with chronic disease (rheumatoid arthritis and ulcerative colitis) as well as aged individuals. Many of the stimuli that we plan to use in mice can also be used in humans. This brings a great deal of complementarity to our animal research programme. Its also directly reduces the number of animals that we will be using as much more work can now be done in humans. In addition, to novel human models our *in vitro* expertise utilises live human cells to assess the effects of various agents on expression and/or release of inflammatory mediators/modulators and functional assays such as migration, proliferation and phagocytosis (all essential facets of the inflammatory response). Since molecules shown to be inactive in these *in vitro* assays are not examined further *in vivo*, these wider activities can be seen as a means of reducing the numbers of animals utilised in the project. Finally, the project utilises a wide range of inflammatory models and models of arthritis each of which in turn incorporates a variety of different treatments. The models are described in more detail below, but the options available mean we can select the most appropriate and least severe model for the question being addressed and alter some of its properties to allow, for example, the establishment of immune and non-immune variations.

Refinement

Explain the choice of animals 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.

Refinement

Based on years of research using rodents as well as recent similar research on humans, we are surprised and equally heartened to see that many similarities seen in mice are also shared in man. This means that mice are a very good choice to use for my research. The advantage over humans is the obvious ability to genetically manipulate pathways in them to bring mechanistic insight into our findings. Nonetheless, by carrying out more and more research in humans while comparing and contrasting the outcome with equivalent data sets obtained from mice, **I am convinced that we can substantially reduce the numbers of rodents used in biological research over the next 20 years.**

We have extensive experience with all experimental models listed in this licence. Nonetheless, as these are inflammatory models they intrinsically carry with them an element of pain, which will be controlled using analgesic regimens providing these do not interfere with the aims of the experiment and are in agreement with the Home Office Named Veterinary Surgeon and in accordance with the Home Office Project License under which this work will be performed.

PROJECT 170

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	GLUTAMATE RECEPTOR ION CHANNELS AND SYNAPSE DYSFUNCTION
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The normal function of our brains depends fundamentally on the ability of nerve cells to communicate with each other at connections called synapses using glutamate receptor ion channels (iGluRs). Recently, a large number of mutations have been discovered in the genes of iGluRs. Some of these mutations cause mental and seizure disorders. However, many others, called polymorphisms, occur in healthy individuals. These genetic changes modify critical parts of the iGluR protein but their effects on the function of iGluRs and synapses are unknown. We need to understand how these genetic changes affect synapse function, cause mental and seizure disorders and modify the action of drugs targeting iGluRs. The objectives of this project are:

1. To identify genetic changes in iGluRs that have potentially significant impact on:
    1. the ability of iGluRs to function normally
    2. the effectiveness of iGluR-targetting therapeutics
2. To determine how human genetic mutations in iGluRs linked to disease affect the function of receptors at synapses
3. To determine how more frequently occurring genetic changes in human iGluRs affect them as therapeutic targets.

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 results of this project will help us to understand what characteristics of iGluR functions are critical for normal excitatory synaptic transmission. This project has two likely clinical benefits. First, it will detail molecular disease mechanisms that could ultimately support genetic counselling of patients with iGluR-linked brain disorders

including some cases of intellectual disability and epilepsy. A growing literature indicates that genetic mutations in iGluRs contribute to these devastating diseases. In fact, iGluR mutations have been discovered at an average rate of more than 10 new familial or de novo cases per year since 2007. Second, the project will evaluate the effectiveness of iGluR-targeted therapeutics. Drugs acting to modulate iGluR receptor function continue to be of intense interest in drug discovery. For example, the Sussex Drug Discovery Centre (SDDC) at the University of Sussex (UoS) has received a £4 million grant to develop AMPA receptor potentiators (PAMs) for the restoration of glutamatergic system function in schizophrenia. Furthermore, the AMPA receptor antagonist Fycompa (perampanel) is now used in the clinic to treat seizure disorders. In this proposal, investigating how SNPs in human iGluR receptors will affect the action of iGluR targeting therapeutics could guide personalised or stratified medicines for the treatments of these disorders.

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

Approximately 1500 genetically altered (GA) mice.

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

The expected severity is mild. The GA mice have no detectable phenotypes. Animals will be culled by a Schedule 1 method for experiments.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Because there are currently no viable, alternative experimental models of non-animal origin that are suitable for studying the effects of human genetic changes on synapse function in intact nervous tissue. We will place a lot of emphasis on using *in silico* and *in vitro* approaches to identify only the most worthwhile candidate genetic changes to study using animals.

We will explore alternative genetic engineering tools to replace native iGluR subunits with genetically altered iGluRs without the use of GA animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will reduce the number of mice used by:

1. preparing as many healthy slices (8-10) from each mouse pup (aged P6-8) as possible
2. breed the mice to homozygosity to increase the effect size and thereby reduce the number of mice required for adequate statistical power
3. maintaining balanced sampling and using efficient experimental design.
4. managing the colonies to produce only what is necessary for the experiments in this project without breeding animals surplus to requirements

Refinement

Explain the choice of animals 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.

Refinement

Animals will be provided species specific enrichment and maintained according to best husbandry practise. Subject to the health status of mice entering the facility, animals bred under this licence will be specific-pathogen-free (SPF) and maintained in individually ventilated cages (IVC). In most instances, mice will be housed with companions (typically 2-4 animals per cage). Animal husbandry is regularly reviewed by our NACWO and any steps to improve it are welcome for the mice bred under this licence.

For the most part, we will use inbred GA mice for this project that are primed for knocking-out the corresponding iGluR subunit(s). Unlike there germline knockout counterparts, these mice have no phenotype during routine breeding that segregates with mutant allele. Therefore, the majority of the animals are not expected to show signs of adverse effects that impact significantly on their general well-being. Very rarely the severity of any incidences may be such that the humane end points may be reached. Mice will be killed if they are aged (>12 months) or show signs of ill health. In addition, any adults that loses 10% of its body weight when compared to age matched controls will also be killed.

PROJECT 171

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The testing of osteochondral scaffolds in sheep
Key Words	Joint repair, chitosan, cartilage, sheep
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

A cost-effective new implant has been designed to better treat localised lesions in the knee joint. This project will test this new material in a sheep joint in order to determine its safety and performance *in vivo* before it can be considered for use in the clinic.

This project will test the implant's safety and efficacy to repair a lesion in the knee joint. The performance of the implants will be tested over a period of 16 weeks and compared to an existing product already in clinical use.

The main objectives are-

- whether the new implants are safe, well tolerated and do not cause any inflammation
- does the use of the implant show better repair of the damaged site and less pain
- what is the quality of the repaired tissue produced at the implant site

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 aims to improve treatment outcomes for patients suffering joint damage by introducing a novel clinical proposition. Regenerative scaffold therapies, such as this material, are promising alternatives to traditional treatments for all osteoarthritis patients as they have the potential to enhance repair and ultimately restore healthy tissue. If successful, this cost-effective material could be available to treat large numbers, avoiding a delay in treatment and hopefully a total joint replacement in the future.

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

38 sheep over 5 years

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

We expect that the animals will be slightly lame for a couple of days and then will have no adverse effects of the surgery. Sheep will be monitored and the NVS contacted if an animal is experiencing uncontrolled pain. Animals will be humanely killed to allow us to harvest the knees and investigate the amount of new cartilage and bone that may have grown into the defect site. We will use magnetic resonance imaging (MRI), micro-computed tomography, immunohistochemistry and histology to look at the quality and the amount of tissue regeneration.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Cell based studies are useful to look at how cells will behave in contact with the scaffold, however cell assays alone cannot adequately model the complete array of effects important in cartilage repair. Using *in vitro* experiments we have shown that cells were viable, proliferated, and were evenly distributed throughout the scaffold. Predictions of degradation rates and porosities have been obtained from these studies; however none of these assays can adequately model the *in vivo* joint environment of a whole animal.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will create two defects in one leg to reduce the overall number of animals used. Statistical analysis has been employed to calculate the minimum number of animals we could use and still have a scientifically relevant study. This value has been calculated at 6 defects per experimental group. Data will be analysed using a suitable statistical package and statistical tests, for example one way analysis of variance and post-hoc testing. All experiments will be conducted in a manner that will allow high quality publication.

Refinement

Explain the choice of animals 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.

Refinement

Small animals such as mice, rats and rabbits do not adequately recreate the situation of a human knee, so to get valuable information from such a project it is necessary to carry out this study in sheep as it is an accepted model for the size and morphology of the human knee.

All surgery is carried out aseptically in dedicated facilities with experienced staff, and all animals will receive pain relief during and after surgery. Antibiotics will also be given to prevent infection. Sheep will be group housed after fully recovering from the anaesthesia. If an animal is unable to stand 6 hours after surgery it will be killed to prevent suffering. Any animal which is lame will receive additional pain relief and be closely monitored for 3 days, and if showing no signs of improvement will be killed. A scoring system will be used to monitor the animal’s wellbeing after surgery.

PROJECT 172

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Distinct subpopulations of serotonin neurones: physiology and pathology
Key Words	serotonin, brain function, behaviours, neurosychiatric disorders
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

It is known that neurones (nerve cells) in the brain which make and release serotonin (serotonin neurones) are involved in mediating many behaviours and functions including sleep, feeding, mood and memory. We also know that in neuropsychiatric disorders (including depression, anxiety, psychosis, drug addiction, eating and sleep disorders, and learning and memory difficulties) serotonin neurones may not be functioning normally. The treatments currently available for these disorders and symptoms don't work very well. Designing new therapies relies on scientists having a better understanding of how the brain works normally, how and why it can go wrong, and how treatments can make the brain work normally again.

Recently it has become clear that not all serotonin neurones are the same. They contain different chemicals and proteins and most probably function in different ways. The purpose of this project is to find out about how these different types of serotonin neurones work, which parts of the brain they are in and which behaviours and functions they are involved in. We also want to find out how the different serotonin neurones might be affected by risk factors for psychiatric disorders like stress and also how they might be affected by treatments like antidepressant drug.

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 potential benefits of the project are that we will get a better understanding of how the different types of serotonin neurone in the brain work to control our mood and our behaviour. We also hope to find out how factors like stress affect the way these neurones work, how they can lead to neuropsychiatric symptoms and how we can reverse these effects. In the end we hope that through our work we will be able

to identify targets for new medicines and other treatments for neuropsychiatric disorders.

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

We will use mice in our project. Over the 5 years of the project we will use around 600 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?

In order to investigate our research questions • Some genetically altered (GA) animals will be cross-bred so that they have abnormal pieces of DNA in some of their cells. This won't cause them any ill effects but will allow us to identify the different types of serotonin neurones. • Some GA animals will be given a drug to activate the genetic modification. The drug will be injected or might be given as a pellet implanted under the skin. The drug is known to make animals feel generally unwell so the level of severity is 'moderate'. We will chose a dose which minimises this but still causes the effect we want. • Some GA animals will have very small volumes of harmless virus injected into the brain. For this they will be anaesthetised and a hole will be drilled in the skull. A needle will be used to inject the virus, the scalp will be stitched up and the mice will then be allowed to recover. During recovery they will be kept warm and given a pain killer. This procedure is 'moderate'. Animals might experience mild pain after the procedure but we will give appropriate pain killers. It is possible that some animals will get an infection in the wound or will have a bad reaction to the anaesthetic or injection, but this is unlikely. • Some GA animals (including some which have had virus injected or have been given a drug) will have their behaviour measured in their own cage or in a special arena or maze. Sometimes we will use tasks which involve food rewards, in those cases it might be necessary to restrict food beforehand so that animals will complete the task. In some cases animals will be injected with a drug during the behavioural measure. These procedures are 'mild' and are unlikely cause adverse effects. • Some GA animals (including some which have had virus injected or have been given a drug and have had their behaviour measured) will undergo procedures under general anaesthetic from which they will not recover. These procedures will be used to measure brain activity or brain chemicals or to preserve the brain tissue which will then be used for further study. These procedures are 'non-recovery' no adverse effects are likely. • Some GA animals (including some which have had virus injected or have been given a drug) will undergo stresses like having their bedding wetted, being exposed to a larger unfamiliar animal, or being isolated. These procedures are quite mild and are unlikely cause adverse effects beyond mild stress. Other animals will be treated with medicines or hormones some of which may cause weight loss and general ill health. Often we will have to inject the animals to give the medicines which might cause some irritation or soreness. These adverse effects may be moderate. After these

procedures, animals will have behaviour measured as described above and/or will undergo procedures under general anaesthetic from which they will not recover. At the end of the studies, animals will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Our research concerns specific populations of serotonin neurones and their functions which include mediating complex behaviours. We are also interested in understanding the impact of the internal and external environmental on these neurones. Whilst research in humans is possible, there are both practical and ethical issues which make this impossible. Humans cannot be genetically altered so we have no way of identifying the neurone types we are interested in. It is also impossible for us to examine their brain function in detail while they are alive. We cannot stress people or ask them to take untried treatments. To find out about serotonin neurones and neuropsychiatric disorders and symptoms and the effects of stress, medicines, hormones on these neurones we must use experimental animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

It is important to use large enough numbers of animals so that if there is an important biological effect, we will be able to detect it. Using knowledge about how variable our measures are will calculate the numbers of animals needed to see important effects. We can reduce variability and so reduce numbers of animals needed by doing our experiments very carefully, using animals of the same gender and age, and carefully controlling the housing conditions of the animals. Often we can also make more than one measure in each animal which reduces the overall numbers needed.

Refinement

Explain the choice of animals 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.

Refinement

For our studies we will use mice. We already know a lot about their brains and behaviour and know that in many ways they are very similar to man. Mice can be genetically altered so that different brain cell types can be identified and their function probed. Using GA mice is the only possible way to address the questions we have about different serotonin neurones.

In all experiments we will ensure that animals are handled by experienced and skilled persons and that trainees are closely supervised while acquiring the necessary skills. We will use appropriate anaesthetics and analgesics for surgical procedures. All animals undergoing treatments will be monitored to ensure they are well and are not experiencing adverse effects. We will ensure that we choose doses of drugs and give them to the animals in such a way to minimise the stress to the animal. Where animals have surgical procedures from which they will recover, surgery will be done under sterile conditions and the animals will be carefully looked after and given pain relief as necessary as they recover

PROJECT 173

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	MECHANISMS AND FUNCTIONS OF CORTICAL NETWORK OSCILLATIONS
Key Words	Brain, Cortex, Oscillations, Interneuron, plasticity
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

When neurons in the brain become excited, they ‘fire’ electrical impulses, which they rapidly transmit to all their contacts. Listening in on the brain’s spontaneous electrical activity does not reveal a crackle of random firing, but rather rhythmical tunes of synchronized brain activity, whose beats change when you sleep, rest, open your eyes and perform different mental tasks. These brain rhythms are thought to provide the punctuation for neuronal communication, enabling millions of interconnected neurons to effectively talk to, listen and learn from each other. The aim of this project is to determine the mechanisms underlying oscillations in cortical circuits, and their role in neuronal communication and learning.

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)?

A greater understanding of the mechanisms and functions of brain oscillations will help address fundamental questions in neurobiology, such as how our brains can learn and perform complex tasks, and why we sleep. Disturbances in cortical oscillations have been observed in multiple brain disorders, including Parkinson’s Disease and epilepsy, which affect millions of people at tremendous cost to the national economy. We hope to contribute to the basic knowledge base that, in time, shall have a general impact on the fleld, including clinical diagnosis and possible treatment.

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

This project will use wildtype and transgenic mice. We expect to breed 4000 transgenic mice, and use 3000 wildtype and transgenic mice for ex vivo brain slice

recordings, 250 wildtype and transgenic mice for in vivo recordings under terminal anaesthesia, and 370 mice for in vivo recordings during behaviour.

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

The vast majority of the procedures involve breeding, maintenance and recordings/killing under terminal anaesthesia. These protocols inherently involve mild severity, but approximately 1400 mice may receive viral injections into the brain prior to recording (moderate), to enable interrogation of the underlying circuitry. This involves recovery surgery, which has the potential to induce post-operative pain and/or infection. The risk of post-operative adverse effects will be minimised by the use of aseptic technique and further administration of analgesics, and these surgeries are not expected to induce long-term effects on animal welfare or behaviour. The animals used for in vivo recordings may undergo head-restraint and water restriction (moderate). Implantation of a head fixation bar will involve recovery surgery, which has the potential to induce post-operative pain and/or infection. The risk of post-operative adverse effects will be minimised by the use of aseptic technique and further administration of analgesics. Head-restraint has the potential to cause stress. This will be minimised by habituation, and it is expected that the mice will tolerate head-restraint well. The levels of water restriction necessary to motivate performance the behavioural tasks are not expected to induce overt signs of stress or discomfort.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The project addresses fundamental neurobiological principles, which can only be resolved in living brain tissue. The invasive recordings and manipulations of neuronal activity cannot be performed in humans, although we are now attempting to complement our animal studies with experiments in human brain slices, prepared from brain tissue resected during surgery. We also complement our studies with computer simulations.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The use of transgenic manipulations and optogenetic techniques provides us with powerful tools to dissect brain function in mice. The methods and results will be reviewed to continuously to maximise the information obtained and statistical power.

Refinement

Explain the choice of animals 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.

Refinement

The mouse is an appropriate species for studying neuronal signalling and cortical circuit function, because this species is sufficiently close to primates to be of relevance for human biology and disease. Importantly, the mouse is genetically tractable, and thus uniquely suited to experiments using the optical control of neuronal activity to directly probe circuit function and dysfunction.

The risk of distress following recovery surgery will be minimised by the use of aseptic technique and administration of post-operative analgesics.

A relatively small number of animals will perform behavioural tasks under head restraint, with water restriction used to motivate task learning and performance. The use of head restraint is necessary for simultaneous brain imaging, with automated control of stimulus presentation, behavioural monitoring, and reward delivery. Mice as a species can tolerate head restraint well, and extensive handling and habituation will be used to minimise stress. As a desert species, mice also tolerate water restriction well. We will always use the mildest restriction paradigm necessary for the animals to learn and perform the task.

The protocols we use are well established and scrutinised by the veterinary and research communities. We shall ensure that we further improve these protocols in the light of new developments in anaesthesiology, surgery and behavioural training, as more information becomes available.

PROJECT 174

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Evaluation of therapies for lysosomal storage diseases
Key Words	Pompe Disease, Glycogen Synthase, Skeletal Muscle
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall aim of the project is to identify new pharmacological approaches to treat lysosomal storage disorders and more specifically Pompe disease. Compounds are tested for their ability to improve muscle function by decreasing lysosomal glycogen accumulation and potential adverse events in order to provide significant improvement in the quality of life compared to current therapies.

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

The assessment of efficacy in an intact mammalian system is very important in selecting new successful strategies to treat Pompe disease. These development candidates should be highly effective with fewer side effects than current therapies resulting in a reduction in muscle weakness and cardiomegaly and improvements in quality of life of patients.

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

12000 mice and 3000 rats. These are the maximum numbers top be used over 5 years.

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

The majority of studies described in this Licence are well tolerated by rodents. Mice and rats have been chosen because the induction and progression of glycogen accumulation and related perturbations in the cardiac and skeletal muscle have been well characterised and documented in these animals. Genetically altered knockout

mice with this disorder are expected to display reductions in motor function as early as 3 to 4 weeks of age. Marked clinical signs of muscle weakness and muscle wasting are observed when the animals reach 8 to 9 months of age. By this stage it is expected that the end point for the study shall have been reached. Animals may undergo recovery surgery to implant EEG and EMG devices (for the assessment of heart and muscle function respectively) under aseptic conditions. Appropriate pre- and post-operative care and pain management shall be undertaken to minimise post-operative pain and aid recovery. It is not expected that serious adverse effects will occur but any side effects are likely to involve body weight loss and deterioration in clinical signs. Any animals exhibiting such signs will be removed humanely from the study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animal models for Pompe disease are required to assess the effect of a test compound on glycogen accumulation and muscle function (efficacy).

Cell and *ex-vivo* assays can give a good indication of the potential ability of a compound to modulate glycogen accumulation but they cannot reliably predict *in vivo* efficacy on more complex function such as global muscle function and disease progression. *In vivo* models are therefore an absolute necessity to relate *in vitro* data to efficacy in order to predict a potential clinical benefit. In addition, the PK/PD relationship, driven by distribution, metabolism and elimination, cannot be accurately modelled *in vitro*.

Finally, proven *in vivo* efficacy data is a prerequisite of the regulatory bodies who have the authority to approve or reject a new drug application.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Protocols covered by this project licence application are designed to use the minimum number of animals possible.
Tolerability studies are performed with small groups of animals in order to establish the maximum tolerated dose and suitability of dosing regimen prior to larger efficacy studies. Only then, can the more complex *in vivo* efficacy studies commence in the knowledge that the animals are likely to tolerate the compound.

Minimum group sizes for efficacy studies will be calculated using power analysis and will incorporate consultation with a statistician.

The use of techniques that allow repetitive recordings of heart and muscle activity will avoid unnecessary termination and enhance the amount of mechanistic data obtained in a single animal therefore decreasing the number of animals necessary for each particular study.

Refinement

Explain the choice of animals 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.

Refinement

It is well documented that the genetic deletion of the GAA enzyme in mice induces the accumulation of glycogen in skeletal muscle leading to an increase in cardiac volume and progressive muscle weakness in the limb. These models are based on the gene mutation that have been shown to cause Pompe disease in human patients therefore improving the likelihood of translation of efficacy observed in these models to clinical benefit. Finally, studies in rodents deliver robust, reproducible data and so it is often unnecessary to evaluate efficacy of new compounds in higher species.

The project uses techniques that can also be used in patients during clinical trials such as measurement of muscle and heart function using EMG and ECG respectively. These techniques will provide efficacy data on the key symptoms of the disease and key information on the mechanism of actions of the compound tested that will be directly translatable to the clinical situation.

In addition to tolerability studies, pilot studies may be conducted in a small number of animals in order to refine the parameters and methodology for ensuing studies. These are intended to define the risk/benefit ratio of each procedure to generate statistically significant data whilst causing the least adverse effects on the animals.

PROJECT 175

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanisms underlying circadian clock regulation
Key Words	Circadian rhythms, Light, Sleep, Drug Development
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aim of this project is to understanding the mechanisms by which the 24h body clock or the circadian clock is regulated by environmental signals such as light, food and drugs, and by extension, develop novel therapeutics to modulate circadian rhythms.

The main objectives are to:

1) Characterise the molecular mechanisms by which the circadian clock is regulated by environmental signals such as light, food and metabolic state.

2) Determine how these signals regulate circadian physiology and behaviour

3) Determine the therapeutic potential of novel drugs targeting the above mechanisms in normalising circadian disruption and associated disorders

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)?

Circadian disruption is endemic in today’s 24/7 society with over 30% of the population working shifts and large amounts of sleep-wake cycle disruption in the mentally ill and elderly populations. A large section of the population also shows inherent circadian disruption, such as non-24h sleep wake syndrome, which occurs in 50% of the totally blind. Circadian disruption has been unequivocally linked to other disorders such as obesity, diabetes and depression. The findings and developments from this project will lead to new therapeutics to treat these disorders, one of which we are currently developing within this project with an aim to test in human subjects within 2 years.

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

The project will use approximately 5,000-10,000 mice in 5 years.

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

The majority of our work involves non-invasive measurements of physiology and behaviour. This is largely based upon monitoring the activity/sleep or performing behavioural tests in wildtype and genetically-altered mice to determine how they respond to different light/dark cycles in combination with drug administration. This will involve singly housing mice with running wheels, but a small minority of animals will be monitored using more invasive procedures such as telemetry and metabolic testing. These procedures involve surgically implanting devices that either deliver compounds and/or transmit this information, which can result in post-operative pain or discomfort, which will be treated with analgesics. Our work also involves administration of substances to modify circadian or light signalling pathways throughout the body, or more specifically at the level of the eye or brain. This involves injecting drugs or other substances to alter the expression of specific genes into the circulation or specific areas of the brain. This may result in post-operative pain or discomfort, which will be treated with analgesics. In vivo studies are supported by ex vivo work on tissues collected at the end of experiments.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

This work involves the study of complex physiology and behaviour in systems throughout the body. As such, no alternatives to animal models are available. However, we commonly employ cellular clock models to replace the use of animals where possible. Moreover, collaborations with groups studying human subjects and patient groups also replace the use of animals and inform our in vivo work.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Breeding of genetically altered animals will be closely monitored to prevent over-production. Animal numbers will be reduced by careful experimental design (e.g. power calculation). In addition, we routinely archive our transgenic mouse lines to prevent over-breeding. Finally, we regularly employ randomisation and blinding to ensure our results are reproducible, as well as employing within-subjects

experimental designs where each animal can act as its own control. Certain advances, such as in vivo reporter gene imaging assays that allow longitudinal gene expression animals is freely behaving animals will be tested and will greatly reduce animal numbers.

Refinement

Explain the choice of animals 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.

Refinement

A primary aim of this project is to measure physiology and behaviour over multiple days without disturbing the animals. As such, we use a wide range of non-invasive methods, including measuring activity using running wheels, which provide environmental enrichment to the animals.

The majority of substances tested are approved drugs, deemed safe in man and therefore well-characterised in animals.The best routes of administrations will be determined through the course of the experiments, and advances such as microinfusion pumps will be tested.

In addition, technological advances that enable more refined measures of circadian function such as in vivo luciferase imagers and thermal imaging cameras will be tested.

The animals’ wellbeing will be monitored throughout, and appropriate interventions such as the provision of analgesics, wet food and environmental enrichment will be provided.

PROJECT 176

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Neuropsychology of psychiatric disorders.
Key Words	Psychiatric disorders; animal models; learning, memory, neurobiology
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Psychiatric disorders can be described or considered as aberrant forms of learning and memory. Our project aims to understand the environmental and neural bases of learned behaviours that are related to psychiatric disorders, so that we can develop behavioural and pharmacological treatments for these disorders. We will use pre-existing behavioural protocols and we will develop new ones. Once the behaviour has been established, we will map the neural bases of these behaviours, and then causally establish the relationship between neural function and behaviour. With this knowledge, we will develop behavioural and pharmacological treatments for psychiatric disorders.

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)?

Psychiatric disorders represent a big burden to society not only because of their costs, but also because of the resulting suffering on patients and relatives. We wish to understand the environmental and neural causes of these behaviours in animal models where we can causally interrogate neural function, to then develop neural and behavioural interventions to alleviate these behavioural disorders.

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

We anticipate that we will use fewer than 2000 rats over 5 years.

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

Because we model psychiatric disorders, animals will experience anxiety. As these psychiatric disorders cause distress in humans, some of our behavioural preparations may induce mild pain or hunger (food restriction). Some surgeries induce permanent lesions in order to causally understand the neural basis. However, incidences of adverse effects during/after surgery are minimal. Animals are monitored for signs of pain, and if we can't alleviate these in consultation with the named veterinarian, we will euthanize the animal. Some animals that undergo surgery may suffer pain discomfort associated with the surgery. The level of severity is not expected to go beyond moderate.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animal models allow for a causal-level analysis of the behavioural and brain mechanisms underlying different behavioural protocols with relevance for psychiatric disorders. Although we are pursuing formal modelling of such processes, at this stage we need to generate data to better understand such models before we can replace them with formal simulations.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The applicant teaches at University level experimental design and statistical analyses. The experiments that will be run will make use of mixed designs (or fully within subjects – that use the same subject as experimental and control) hence use the least number of animals possible. This will be further achieved by the use of statistical tools that allow to estimate the minimum number of subjects needed to test a hypothesis based on previous research.

Refinement

Explain the choice of animals 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.

Refinement

Rodents have been used in research for over a century and hence their anatomy, behaviour and husbandry are well known. We use state-of-the-art behavioural

protocols in which the duration of the procedures and the amount of discomfort are minimized. The procedures proposed in this PPL are well known to the applicant and the scientific community, and hence measures are routinely taken to ensure both the animal’s wellbeing, and minimize suffering. Careful monitoring of animals by suitably trained staff will ensure the welfare of the animals and minimize any adverse effects. In the presence of pain, analgesics will be used.

PROJECT 177

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mitigation of obesity by nutritional interventions
Key Words	Obesity, natural products, plant chemicals
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Obesity, diabetes and cardiovascular disease are consequences of the modern food environment. Highly palatable fat and energy rich food are widely consumed. Reformulation of foods with health beneficial natural ingredients would provide a route to improving public health, reducing human suffering (due to complications arising from obesity) and reducing the costs of the public health services.

The proposed project builds on previous experiments in which we have discovered that nutrition during early life and plant derived supplements can generate a partial protection to obesity. The objective of the current proposal is to find out how this protection is achieved and how these physiological mechanisms can be used for the development of food products with health benefits.

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 long term objective of the proposal is the development of food products which assist healthy weight management and lead to an improvement of metabolic health.

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

The proposal will utilise a number of mouse systems to explore the mechanism of two interventions which provide a protection against obesity. The total number of mice will not exceed a total of 2800 over the lifetime of the license (5 years).

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

The proposal will utilise non transgenic and transgenic animals. None of the genetic changes is the transgenic animals have any adverse or distressing phenotypes. The

experiments are all feeding experiments in which mice are fed defined diets and are analysed for the effects that the diet has on their weight, body composition (by MRI scanning) or glucose metabolism (intraperitoneal glucose tolerance test). The typical time span for the feeding experiments will be in the range of 3 to 5 months. Almost all mice will be killed by a schedule 1 method at the end of the experiment and appropriate tissues will be analysed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Common lifestyle diseases including obesity, diabetes and cardiovascular disease are a major drain on the public health system. In order to investigate the physiological mechanisms which cause these diseases it is necessary to examine the interplay of several organs including e.g. liver, pancreas and adipose tissue. In the context of this project we are aiming to compare results from mouse model systems of diet-induced obesity with results from sophisticated tissue culture studies (which include different growth matrices and different cell types). The long term goal is to replace as many animal experiments with cell culture studies as possible.

However in addition to animal cells, bacteria play essential roles in the digestion process and in the transformation of food components. This interaction between animal and microbes is difficult to model in cell culture at present. However the planned experiments will assist in defining the role of phytochemical components contained in the diet and the animals and the microbes more closely and may in the long term help to replace animal experiments with in vitro analyses.

One further aspect which we plan to study is the role of early life nutrition in adult health outcomes. This involves the modification of nutrition in early life and the monitoring of health outcomes in adulthood. This process involves many parameters (e.g. number of cells in a tissue, number of blood vessels in a tissue) which cannot be adequately mimicked in cell culture. Our approach seeks to identify the overall biological and medical outcomes of early nutrition in animals and then to focus on specific aspects of the underlying mechanisms in cell culture (e.g. regulation of critical genes).

The proposal requires the breeding of some transgenic mouse strains. However none of these strains have any detrimental phenotype which would lead to distress in the animals. E.g. we are using a transgenic model in which growth in early life as attenuated. Offspring which have undergone this attenuated growth are partially protected against obesity. Growth in early life and long term health consequences cannot be mimicked in cell culture systems.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Using the appropriate number of animals in experimentation is very important. A sufficient number of animals to measure a health effects reliably is necessary. If the results obtained from the experiment are not statistically relevant the animals would have been used in vain. On the other hand it is also important not to use too many animals. Each animal in an experiment generates significant costs and requires a significant input of time by animal staff and researchers. In addition it would lead to the breeding of an unnecessary number of animals. The number of animals used was therefore carefully calculated based on our previous experience (with regards to the effect sizes of the interventions) and thorough statistical advice. The minimum number of animals required to produce a valid result will be used.

Refinement

Explain the choice of animals 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.

Refinement

Mice (especially the C57B/6 strain used in the experiments) have been shown to be show similar responses to high fat diets as humans (slow and steady increase in weight, development of glucose intolerance, hypertension). In order to obtain the best results from animal experiments it is important to maintain mice in such a way that they experience no stress. The mice will be housed in enriched environments suited to their requirements so that they can exercise their normal behaviour. As far as possible mice will be housed in groups as this is how they live in the wild. Male mice will only be grouped together before puberty so that they do not fight with each other to establish a social ranking structure, In the proposed project most of the procedures experienced by the mice will be mild (such as weighing, blood sampling or Echo MRI scanning). All procedures will be carried out by experienced staff. Care will be taken that most procedures will be carried out by the same staff members, as mice are able to identify humans and become accustomed to being handled by the same person.

PROJECT 178

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Stromal function in the tumour microenvironment
Key Words	Tumour microenvironment; Stroma; Immune; Immunotherapy; Precision medicine.
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Cancers have developed many ways in which to prevent our defences, that is our immune system, from destroying a tumour. New therapies that ‘kick-start’ our immune system back into action are proving promising, but these still only work on some patients in a few cancers. A tumour is much more complex than a collection of cancer cells. Many other cell types make up the tumour – these cells support the tumour in many ways and are known as the stroma. These stromal cells may help a tumour by acting on our immune system to prevent it from working properly, or by attracting bad immune cells. There is still very little known about how the stroma may do this, but from the evidence we do have, it is likely to have a key role. This project aims to identify how stromal cells act upon the immune system as a tumour develops, and to use this knowledge to enable us to better and more specifically treat 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)?

From this project our primary benefit will be the increased understanding of how supporting cells found in a tumour act, specifically how they work to switch off the immune system. These findings will provide immediate benefit to the research community sharing potential mechanisms and features that could be used for development of new or improved treatments that use our immune system to target a tumour. New mouse models and experimental systems developed will offer potential to benefit other scientists, and the knowledge we gain will begin to benefit clinicians, Biotech industries with drug discovery pathways, hopefully being initial steps towards the patient benefiting.

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

We will use a maximum of 6300 mice in this project covering breeding and experimental protocols over a period of 5 years. Mice represent the least complex system relevant to human disease and the complex nature of cancer, especially the complexities of tumour immunology. Genetically altered mice develop tumours with similar features as human disease, share patterns of spread and contain a functioning immune system that can be compared with human.

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

Animals under the authority of this PPL will develop tumours that have been induced by a variety of methods such as cell implantation, inducing genes in genetically altered animals, using chemical carcinogens or using viral vectors. Tumour growth will be measured and blood samples may be taken. To investigate how stroma contributes to tumours, tumours may be imaged on a microscope where substances such as labelled cells, blocking antibodies or cell tracers may be administered by a range of routes such as intratumoural injection or application to skin. To help identify key populations, animals may also be irradiated to remove cells which are replaced with altered cells to allow us to identify those needed for tumour development. In the majority of cases, tumours will not significantly impact animal welfare or normal behaviour and will be classified as mild. Rarely, mice may develop more clinical signs such as tumour ulceration or weight loss. If these are seen, mice will be killed immediately to limit any suffering experienced. However, in the vast majority of cases, our endpoints occur before any of these adverse effects have the chance to develop thus most animals be experience mild severity in their lifetime, and will not exceed a moderate severity limit.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Tumours contain tumour cells, but they are in fact much more complex systems including fibroblasts, blood endothelial cells, lymphatic endothelial cells, pericytes, adipocytes, smooth muscle cells, fibroblast and immune cell populations all of which interact, adapting as a tumour develops and evolves. We work routinely with in vitro models, making them as complicated and accurate as we can to generate as much information as possible with regards to the tumour microenvironment. The model systems we use aim to complement and help design mouse experiments appropriately. They help to identify key populations to investigate in mice, doses,

potential responses to therapy before using mice. They also critical for us to home in on specific mechanisms following data generated in mice. However, the complexity of a changing tumour cannot be fully recreated in a plastic culture vessel. To do this we require a living system with aspects of the immune system comparable with humans. Such a system does not exist in non-protected alternatives.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Before conducting any experiments, we perform statistical analyses to determine the fewest mice needed to produce useful data. We extensively use complex in vitro systems as a means to generate data without animals, but these are still not able to recreate a living animal. Thus we need to use animals. However, from each mouse we will collect multiple tumours and associated tissues i.e. lymph nodes that are analysed using multiple methods. Tissue from the mice also help to make cells which are further used the in vitro systems described above, which complement and help refine our research design. Moreover, the genetic models used can develop multiple tumours at differing stages per mouse as occurs in human disease. These approaches allow us to gain as much information from each animal as possible and reduce the numbers we need to use.

Refinement

Explain the choice of animals 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.

Refinement

We need a model with a complex, functional immune system as is found in humans, and which is lacking in lesser species. Thus, mice represent the least complex system that possesses the parameters needed to yield meaningful data translatable to human disease. We have extensive experience in mouse tumour models and work closely with staff at the animal facility. We have refined models so that animals largely develop superficial tumours and experience very few clinical signs. This means our experiments end before adverse effects can develop. As animals are monitored very closely, by well trained staff, any welfare concerns raised can be dealt with rapidly. Mice are also housed in social groups and are given multiple bedding types for nesting, chew sticks, fun tubes and platforms to provide a rich living environment.

PROJECT 179

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Pharmacology and physiology of CNS neurotransmission
Key Words	Pharmacology, Physiology, Brain
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Psychiatric illnesses such as depression, anxiety and schizophrenia are distressing, seriously disruptive of life and, for a significant number of sufferers, lead to loss of life through suicide. Despite this, even the most modern psychotropic drug treatments do not achieve useful therapeutic effects in a third or more patients, often have a delayed onset of action of several weeks, and frequently cause debilitating side effects such as sedation, weight gain (and through this metabolic disorders such as diabetes) and peripheral disturbances that many patients find intolerable.

One of the main reasons for all this is that we still don’t fully understand how the drugs work in the brain or what causes the mental illnesses that they are aimed to treat. In particular, there is a need to increase knowledge of the neurotransmitter systems that underpin psychiatric symptoms such as disturbed emotional and cognitive behaviours, and their subsequent relief by treatment. With this knowledge, drug treatments could be developed rationally rather than by empirical means as at present, and treatments could be prescribed with a prior knowledge of whether they will work in a particular patient or not.

The overall purpose of this programme is to utilise animal models to gain new knowledge on the pharmacology and physiology of neurotransmitters in the brain, and to use this knowledge to help make advances in the treatment, prevention and detection of psychiatric disorder. Our programme has 4 specific Objectives (with examples of key questions):

1) To identify novel mechanisms of neurotransmitter function.

2) To identify how neurotransmitter activity changes during emotional and cognitive behaviour.

3) To study the effect of neurotransmitter manipulation on emotional and cognitive behaviour.

4) To develop novel neurotransmitter-based therapeutic strategies.

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

We expect our research programme to have the following benefits: i) Our data will to contribute to the understanding of the basic pharmacology and physiology of complex brain circuits and how changes in these circuits cause changes in behaviour. This new understanding will be of interest to a large community of researchers in many disciplines including neuroscience, pharmacology, psychology, psychiatry and computational neurobiology. ii) Our data will generate models that are aimed to develop and predict the design of future drug therapies that will be more effective than treatments of psychiatric disorder and with fewer side effects. This understanding and increased capacity to predict the future will benefit industry that is investing in new drug therapies. Also, it will be helpful to clinicians who are treating patients and developing prevention strategies. It will also be of value to patients and their carers themselves, who are trying to understand and cope with psychiatric disorders. iii) Our data will reveal new information on the genetic factors that put individuals at risk of developing psychiatric disorders such as depression and anxiety. This information will be helpful to clinicians who are treating patients and detect those at risk. It will also be of value to patients and their carers themselves, who are trying to understand and cope with psychiatric disorders. iv) Through collaboration with computer scientists our data will generate computational models of the functional connectivity of key microcircuits relevant to psychiatric disorders. These models will be used to make further predictions that we can test and eventually get a much more complete explanation of how these microcircuits are changed by genetic and environmental risk factors and acted on by drug therapies. These developments will have benefit the 3Rs in terms of reducing and replacing animal use.

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

During the 5 year duration of this research programme we expect to use approximately 2,220 mice and 650 rats per year.

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

The experiments in our programme of work with be classified as overall moderate severity. Our main experimental approach is to manipulate neurotransmitters in the

brain in a number of complementary ways including the use of animals with key neurotransmitter-related genes modified, administration of drugs and use of optical stimulation to control neurons with a high degree of control. Outcome measures will include molecular and cellular transmitter markers in post-mortem brain tissue as well as measurements of neurotransmitter function in in vitro models. Other outcome measures will include measurements of neurotransmitter release, the electrical activity of individual neurons and neural networks. Behavioural analysis will also be carried out, and include measurement of natural emotional and cognitive responses that are highly relevant to symptoms of psychiatric disorder. Some of these approaches involve the surgical preparation of animals under general anaesthesia, for example to deliver genes to the brain or for intracerebral implantation of electrodes for electrophysiological recordings. We will use appropriate surgical methods (aseptic conditions, short surgery time, temperature control) and post-operative care (easy access to food and water, pain-relieving drugs) to maximise recovery and minimise adverse effects. Adverse effects associated these surgical approaches are rare but range from wound breakdown and infection to cerebral oedema which may result in neurological signs. Animals will be monitored daily post-surgery to detect signs of adverse effects before they fully emerge, and thereby allow us to adjust our experiments to reduce their frequency and severity. Some animals will be treated with pharmacological agents. The vast majority of these agents will have known pharmacological effects and proven safety in animals. Some of the agents may cause short-lasting behavioural stimulation and will be administered using dose regimes that produce effects of the smallest magnitude and shortest time appropriate to the experiment. In rare cases pharmacological agents may cause short-lasting adverse effects such as malaise, loss of body weight, and an appearance of general neglect. Animals administered pharmacological agents will be frequently monitored and weighed, and if such adverse effects are observed they will be resolved by adjusting the dosing regime. In certain behavioural tasks animals will be placed on scheduled access to food to ensure that they are motivated to perform the task. These animals are generally healthier than animals with unrestricted access but we will carefully monitor for excessive weight loss using quantitative scoring sheets with endpoints that trigger supplementary feeding to return weight to acceptable levels. At the end of the experiments, or when adverse effects are predicted to exceed moderate severity, animals will be killed by humane methods such as overdose of general anaesthesia.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We need to use animals in our research programme for several reasons:

i) Our studies on neurotransmitters are focused on complex neural pathways that cannot currently be modelled without animals.

ii) Emotional and cognitive responses are complex behaviours with human correlates, and these cannot be studied in non-sentient animals.

iii) The influence of genetic and environmental (eg. developmental) factors on emotional reponses and cognition cannot be modelled in vitro or by computers.

iv) By using animals we can administer pharmacological agents at doses and over intervals that have direct clinical relevance. The relevance of drug doses in in vitro preparations to doses experienced by patients is often uncertain.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will minimise the number of animals by optimising our technological approach and experimental design through the following measures:

i) We will use multiunit recording methods that have the potential to detect the firing of many tens of identified neurons in a single animal. This will reduce the number of animals required, and at the same time generate large biological datasets that are required to capture the complexity of the neural circuits that we are studying.

ii) We will share our datasets with collaborating computer scientists who will develop computational models of key neural networks to aid further hypothesis development and prediction testing. This will directly reduce animal use.

iii) We will use well-managed and conservative genetic mouse breeding strategies and use in experiments of both male and female mice, as well as heterozygote mice where appropriate.

iv) We will use appropriate controls to prevent false positive/negative results, eg. use of drug vehicles, an inactive drug of similar chemical structure, or pharmacological blockade to confirm specificity of action. Rather than repeating controls, we will use "rolling controls" for experiments spread over time.

v) We will use methods of statistical analysis and randomised, blinded experimental designs which maximise the power of the data and minimise the group size. Where necessary we will use alternative methods of statistical analysis to minimise the need for a repeat experiment and/or maximise the conclusions drawn, and power calculations to establish the minimum number of animals required to achieve a meaningful result.

vi) Good surgical methodology and practice will be used to minimise loss of animals by maximising chance of recovery, eg. asepsis, use of short acting anaesthetics, minimal surgery time, thermoregulation, and postoperative care and analgesia.

vii) Pilot experiments will be used before embarking on full-scale studies, and literature searches will be carried out to avoid replication of experiments already published.

viii) Where possible, in vitro experiments will be used to generate models and hypotheses to guide in vivo experiments, and thereby reduce animal use.

Refinement

Explain the choice of animals 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.

Refinement

Experiments will be designed to avoid animal suffering in a number of ways:

i) We will use appropriate surgical methods and post-operative care, including the use of pain-relieving drugs, to maximise recovery and minimise adverse effects.

ii) Drugs/vehicles will be administered, in volumes and routes that are the least harmful and appropriate.

iii) Many drugs selected will be already proven to be safe for administration to animals and humans. Some drugs will have well known psychotropic effects, and these agents will be administered using dose regimes that produce effects of the smallest magnitude and shortest time appropriate to the experiment. In cases where knowledge of the pharmacology is incomplete, we will commence with pilot studies using low doses.

iv) We will use literature and other databases to aid selection of drug treatments or lesion/stimulation sites that produce optimal effects and minimal adverse effects. Treatments with this potential will be detrimental to our studies, and avoided.

v) Pilot experiments will be used before embarking on full-scale studies, for example when using drugs, lesions or genetic constructs of uncertain potential to induce adverse effects.

vi) To minimise adverse effects of neurotoxic lesions, toxins will be selected on the basis of high chemical specificity, and lesion sites will be discrete and unilateral when bilateral is not critical to the experiment.

vii) We will apply newly developed optogenetic and chemogenetic methods to stimulate/inhibit neural pathways. These approaches can be advantageous over

traditional electrical stimulation methods as it targets specific neurones within a nucleus rather than the whole population, and more refined that chemical toxins which will only be used when optogenetic/chemogenetic approaches are not appropriate.

viii) We will measure behavioural change using a range of cognitive and emotional tests that have proven value as constructs of cognition and emotion. We will focus on tests of cognition and emotion that have the least risk of pain, suffering, distress or lasting harm, and the highest translational value, ie. there are equivalent models in human.

PROJECT 180

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Brainstem circuits controlling gut-brain communication
Key Words	eating habits, gut, brain network, obesity, intestinal health
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The objectives of this Project Licence are centred on the regulation of the gut-brain interaction, and how visceral information are received and computed by discrete groups of nerve cells to maintain health. To achieve this, we take advantage of genetic engineering techniques to precisely interrogate the function and the chemical content of discrete brain cells and to correlate these specific cell manipulations to changes in brain or gut health.

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 benefit of the work will be the advancement of scientific knowledge on the logic by which the brain compute and store messages sent by the gut. These knowledge will be directly relevant for human and animal health and welfare. For example, in humans there is high co-morbidity of obesity, gastrointestinal and psychiatric diseases (i.e. anxiety, obsessive compulsive disorders, and depressive states). Understanding how the communication between the gut and the brain is regulated may have implications for the health and wellness of animals (including humans), in that it will provide an evidence base for the development of novel pharmaceutical, behavioral or nutritional intervention to treat or diagnose brain and gut diseases.

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

This program of work may use 4000 mice over 5 years

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

Mice may be injected with physiological doses of hormone or pharmaceutical agents. This is a mild procedure that very rarely promotes adverse effects. In some cases, treatments may lead to reduced body weight. Some mice may experience surgery. Possible pain is minimised by good surgical and aseptic techniques, suitable anaesthesia, good perioperative care and adequate provision of pain relief. This is a moderate procedure. Likewise, some mice may experience gastrointestinal discomfort when subjected to experiments mimicking the human intestinal bowel disease. All other procedures involve the monitoring of natural behaviour, including eating habits; procedures that never promote adverse effects. Any animal showing any signs of ill health will be closely monitored, receive veterinary treatment or will be humanely euthanized. At the end of testing, mice will be humanely euthanized for tissue and data collection.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animals are the only model for the proposed licence because it is not possible to study gut-brain communication using cultured cells or computer modelling. At present, to understand how these two distinct, yet tightly connected, body districts interact requires the use of live animals. However, the research programme seeks to identify means for replacement by incorporating mathematical modelling when available, and basic knowledge that will be acquired with this program of work also have the potential to facilitate replacement.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Sample sizes will be based on statistical power analysis from several prior experiments and power calculations conducted with a statistician. Sample sizes will be reduced to include the minimum number of mice necessary. Built into the experimental design and dissemination of the results are the ARRIVE guidelines established by the NC3Rs. Measures taken to avoid unjustified duplication of procedures will include close monitoring of literature; conference attendance and discussing current procedures with colleagues and veterinary staff.

Refinement

Explain the choice of animals 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.

Refinement

The mouse is the species of choice. There is a large scientific literature on this species and other alternative models do not allow for precise genetic manipulations of brain cells during enacted behaviour. Refinements for injections include the implementation of scoring sheets and humane endpoints. Pain and suffering is minimised by good surgical and aseptic techniques, suitable anaesthesia, good peri-operative care and adequate provision of pain relief. To prevent duplication of experimentation, scientific conferences are attended and discussion held with colleagues. The scientific literature is continually reviewed and veterinarians consulted for alternative surgical treatments and novel means to alleviate adverse effects.

PROJECT 181

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Translational and Integrative Neuroscience for CNS Drug Discovery
Key Words	Neurodegenerative disease,, Neuropsychiatric disease, rodents, biomarkers, drug discovery
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Neurodegenerative and neuropsychiatric conditions remain a substantial clinical problem for society. More effective, safer treatments are still urgently required to help patients.

This project aims to gain greater understanding of the mechanisms by which CNS disorders arise and persist, so that drug treatments that fulfil unmet medical needs can be identified and developed.

A second critical aim of the project is to determine objective biological markers of CNS dysfunction, such that direct relationships can be inferred between findings in patients and experimental subjects (in this case, rats and mice).

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 greatest success of this project will be to identify a novel treatment for a CNS disorder that provides more effective treatment in partly responsive patients, or provides treatment in conditions that are currently poorly responsive to existing treatments, or is free of the sometimes serious side effects that some existing treatments possess. In this process of drug discovery, insights into the mechanisms of CNS dysfunction will be gained, most importantly with regards to the correspondence of the underlying mechanisms between humans and rats. This work will directly challenge the utility of many different aspects of rodent CNS model research to determine which aspects hold the most predictive validity for findings in humans.

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

Rats and mice will be used for this work over a period of 5 years. Approximately 2000 rats and 5000 mice are likely to be used annually.

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

The basic premise of this project is to use rats and mice to develop models of CNS disorder that relate to the human experience, measure both behavioural and physiological endpoints from these animals, and to determine how novel treatments influence these endpoints. Adverse events can arise from these three main objectives: induction of a model of CNS disorder, measurement of endpoints, and administration of novel treatments. As the principle endpoints for studies of CNS disorder largely involve assessment of behavioural funciton, there is no choice but to use awake, behaving animals in these studies. The main procedures used in this project will be assessments of cognitive function via maze, arena or operant box tests of behaviour in animal models of disease, and how drugs affect performance in these tasks. In some studies, measurement of physiological parameters will also take place via implanted devices or catheters to record electrophysiological signals and/or neurochemical measures or to collect blood or cerebrospinal fluid samples. Adverse effects of behavioural testing will be absolutely minimal, as most endpoints of interest cannot be measured effectively if animals are stressed. Very occasionally (<0.1% incidence), administration of novel compounds can result in unexpected adverse effects that might require animals to be immediately and humanely killed, for instance seizures or respiratory distress. Surgical interventions can cause some level of transient post-operative pain that is effectively controlled by analgesic regimens. Pre and post-operative antibiotic regimens are used to minimise the chance of post-operative complication via infection. Upon completion of study, or before completion of study if humane endpoints have been reached, animals will be killed by a Schedule 1 method. In some studies, animals may be killed by appropriate non-Schedule 1 methods for purposes of collection of tissue samples.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The behavioural and functional endpoints of interest to this programme of work are essentially systems-level experiences that are not currently possible to deconstruct in a meaningful way into lesser parts that avoid the use of awake animals. Further, understanding the balance between efficacy and adverse effects of novel treatments

essentially requires the integrated physiological systems of a whole animal to maximise the predictive validity of the data.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

All studies will follow Institutional guidelines on best laboratory practice, where statisticians will be consulted to guide best study designs.

Poorly performing assays or models showing large variability or inconsistent effects will be halted as soon as this is detected.

For novel drug treatments, pharmacokinetic data and/or measures of target engagement (i.e. how well the drug interacts with its target at different doses) are collected before testing for in vivo analgesic efficacy. This obviates the need for "dose-finding" studies and prevents animals being used for studies where inappropriate doses have been chosen.

Refinement

Explain the choice of animals 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.

Refinement

Rats and mice will be used since there is an abundant database of the behavioural, physiological and pharmacological characteristics of these animals. Rats and mice can reliably model clinically relevant aspects of CNS disorder, i.e. tau or amyloid propagation or cognitive dysfunction. Gross anatomy of structures involved in human neurodegenerative and neuropsychiatric disease structures is broadly comparable to that observed in rodents. As a result, rats and mice aredependable experimental systems for modelling different pain states and predicting clinical analgesic efficacy of test compounds.

While the intent of this project licence is to be able to model different forms of CNS disorder in rodents in an attempt to replicate aspects in humans as closely as possible, the use of each model shall be defined and limited by expected humane endpoints. Any escalation of endpoint beyond these pre-defined criteria would result in immediate killing by a Schedule 1 procedure.

The investigation and quantification of behavioural correlates of CNS disorders requires active, healthy animals if reliable and meaningful results, free of experimental errors are to be obtained. The behavioural endpoints being measured

in this project involve sensorimotor testing, naturalistic behaviour or food-rewarded operant behaviour, none of which can be reliably measured in severely stressed animals.

These endpoints are extremely sensitive to pain, suffering or distress and any abnormal response (e.g. trial omissions, lengthy response latencies) will prompt immediate review, revision and/or termination of the study as appropriate. Elevation of the severity limit of our studies to severe (whether acute or cumulative) at any point in any animal effectively invalidates the behavioural endpoints and the whole purpose of this program of work.

PROJECT 182

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Neurobiology of emotional and cognitive impairments in psychiatric disorders
Key Words	major depression, psychiatry, animal model, emotion, stress
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project aims to further our knowledge about the way the brain functions to control emotional behaviour and cognition, so that new improved treatments for mood disorders can be developed. Our project uses animals to model specific aspects of human psychiatric disorders and their symptoms.

Specifically, this project will:

1. Investigate how mood disorders develop and the underlying biology. This work will help to explain why some people get depressed and how treatments might be improved in the future.

2. Investigate the co-morbidity of emotional and cognitive symptoms in chronic illnesses. We will try to find out why so many patients with long term health issues also suffer with diseases like depression.

3. Develop better methods for studying psychiatric disorders in animals and check that these can accurately mirror aspects of the human condition.

4. Assess the welfare of animals in response to laboratory stress to develop more refined methods.

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 immediate benefit of the work will be to the wider scientific community, whilst in the longer term the work is expected to lead to improvements in clinical treatment. The majority of studies will use a behavioural measure where the animal is trained to perform a task to obtain a food reward. These behaviours are then shaped so that

the animal has to follow a specific rule and use cognitive and/or emotional processes which we can relate to similar experiments in humans. This will provide information which can be used to advance our understanding of how the brain processes emotional and cognitive information, and the mechanisms which regulate these processes under normal conditions and when they go wrong in a disease. As so little is currently known about the relationship between the biological processes in the brain and what these mean in terms of psychological effects i.e feeling sad, the work will provide important scientific advances. In the longer term, the knowledge gained from this work should provide novel drug targets and methods to better treat psychiatric symptoms. Specific benefits and beneficiaries include: Neuroscientists (academia and industry, short term) through knowledge gain Animal behaviour scientists and animal welfare researchers (short term) through validation of improved methodologies and refined techniques Patients, psychiatrists and health care workers providing patient care (medium and long term) through improved understanding of disease biology and better treatments. Public understanding of depressive illness and mental wellbeing by raising public perception that psychiatric symptoms are part of a biological illness.

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

Rats ~ 2200 over 5 years, Mice ~1600 over the 5 years.

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

The animals used in the licence will primarily be used in reward-based behavioural tasks for which a licence is not needed but, they are used in combination with method which can cause pain suffering or lasting harm. For most animals, their experience will involve mild procedures such as injection and exposure to short term stressors. None of the procedures used in the awake animal will cause more than transient pain however, animals may be exposed to multiple stressors or repeated drug administration which can contribute to cumulative suffering. To monitor the impact of the treatments, animals are checked regularly for any signs of the development of abnormal behaviours or evidence of stereotypic behaviours. As most animals (~90%) are tested in behavioural tasks involving reward, we can closely monitor wellbeing and expect animals to only show subtle changes in their behaviour, which can only be detected at a group level. If any animal shows signs of below normal task performance they will be reviewed and the vet contacted. Some animals may be exposed to aversive training methods so that behavioural responses to negative emotions can also be investigated. This will affect only a small number of animals used in the overall programme and will use aversive noise or air puff where possible. If footshock is needed, the intensity will be kept below the level that induces fear and freezing behaviour and animals will normally be able to escape by pressing a lever or moving to another part of the cage. For some experiments,

surgical interventions are required to alter the function of a specific part of the brain or to enable stimulation. This will involve surgery with recovery and will therefore be moderate severity. All animals will be given appropriate pre- and/or post-operative analgesia and careful intra-operative and post-operative care. At the end of the experiments all animals will be killed. This may be carried out as part of the protocol to permit the collection of tissue for post-mortem analysis or using a Schedule 1 method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

These studies require an intact living brain in order to achieve the objectives. In order that the work is directly translatable to the human brain, a mammalian species is also necessary. The majority of the studies described will use rats, as they are the most appropriate species for the achievement of the objectives. Mice are the species of choice for genetic studies so some work in this project will use both mice and rats. A small number of neonatal animals are included in this project. These are animals that will undergo procedures during the pre-weaning period to induce long-term changes in the adult brain. This is necessary as many psychiatric disorders have been linked to insults experienced in early life including pre-birth.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Each of the experiments proposed have been carefully designed to achieve the project objectives whilst ensuring that the appropriate numbers of animals are used to achieve statistical power and validity to the data generated. We have now generated more than 10 years of data using these methods so can accurately predict the variability of the data associated with any given method as well as a meaningful effect size. These factors are included in power calculations such that any subsequent experiment uses the lowest number of animals.

The animal numbers over the course of this licence are based on power estimates, our current funding and projections for future funding over the next 5 years. We also work to an ~80% success rate as not all animals will successfully train in the tasks and reach criterion for inclusion in the experiment.

Refinement

Explain the choice of animals 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.

Refinement

The behavioural tests primarily use positive reinforcement and punishment is achieved through a 'time out' procedure where the animal is unable to obtain an outcome to its responses during this period. In a small number of behavioural tests, an aversive stimulus is used. Mild aversive/stressful stimuli may also be used to manipulate the emotional state of the animals, something which is also necessary to achieve the project objectives. We have refined the techniques to use the minimum level of aversive stimuli necessary to trigger the behavioural outcome we are investigating. This has also meant we have developed husbandry and procedural techniques which reduce stress in control animals helping also to refine the stress/aversion needed for the induction of a depression-like change in the animal.

The surgical interventions use methods which do not cause overt changes in behaviour and post-operative analgesia is provided. Within 48 hrs post-surgery, animals return to normal and are usually group housed.

When we induce a model of disease we will use the least invasive approaches and we focus on social or environmental manipulations or treatments with pharmacological agents. These procedures compare well with factors that have been shown to influence human psychiatric conditions and also induce changes in behaviour that reflect depressive-like and/or anxiety-like behaviour.

In all the protocols, animals may be exposed to mild stressors and/or receive drug treatments which may involve mild discomfort during administration. Animals will be habituated to handling and dosing procedures, and monitored to ensure they are not developing any sensitisation to the procedure. Control animals will also be housed and handled using methods which optimise welfare.

Endpoints: When animals exhibit signs of poor performance in tasks, this is used as an early indication that they are showing signs of ill health and will be discussed with the animal care staff and/or vet and animals removed from the experiment and killed if they do not return to normal behaviour. To monitor the impact of cumulative suffering, animals will be routinely monitored for body condition, weight and the presence of abnormal or stereotypic behaviours including aversion to handling. Together, these measures provide endpoints which are used to limit the overall severity of the protocols.

PROJECT 183

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Evaluation of therapeutic strategies for narcolepsy
Key Words	Narcolepsy, Cataplexy, Orexin
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall aim of the project is to identify new pharmacological approaches to treat narcolepsy. Compounds are tested for their ability to reduce excessive daytime sleepiness and cataplexy (sudden loss of muscle tone) and for adverse events in order to provide significant improvement in the quality of life of patients compared to current therapies.

Narcolepsy is estimated to affect about 1 in 2500 people with 84% of patients still reporting significant symptoms on a daily basis despite treatment. This work is expected to provide new information on mechanisms regulating the sleep-wake cycle, which will be used to identify new therapies with better efficacy and fewer side effects.

The majority of studies described in this Licence are well tolerated by rodents. Mice and rats have been chosen because the sleep patterns and mechanism of sleep disturbances have been well characterised and documented in the literature. It is not expected that serious adverse effects will occur but any side effects are likely to involve body weight loss and deterioration in clinical signs. Any animals exhibiting such signs will be removed humanely from the study.

Narcoleptic animal models are required to assess the effect of a test compound on excessive daytime sleepiness and cataplexy (efficacy). Cell and *ex-vivo* assays can give a good indication of the potential ability of a compound to modulate brain activity but they cannot reliably predict *in vivo* efficacy on complex neurological processes such as sleep/wake cycles. *In vivo* models are therefore an absolute necessity to assess compound efficacy in order predict a potential clinical benefit. In addition, the fate of compounds after administration, driven by distribution, metabolism and elimination, cannot be accurately modeled *in vitro*.

Finally, proven *in vivo* efficacy data is a prerequisite of the regulatory bodies who have the authority to approve or reject a new drug application.

Protocols covered by this application are designed to use the minimum number of animals possible. Tolerability studies are performed with small groups of animals in order to establish the maximum tolerated dose and suitability of chronic dosing prior to larger efficacy studies. Only then, can the more complex *in vivo* efficacy studies commence in the knowledge that the animals are likely to tolerate the compound.

Minimum group sizes for efficacy studies will be calculated using power analysis and will incorporate consultation with a statistician.

The use of techniques that allow repetitive recordings of neuronal and muscle activity will enhance the amount of mechanistic data obtained in a single animal therefore decreasing the number necessary for individual studies.

It is well documented that orexin-deficient mice express narcoleptic episodes, which mirror many of the primary symptoms of the clinical disease. These models are based on the same mechanism that has been shown to cause both cataplexy and excessive daytime sleepiness in narcoleptic patients therefore improving the likelihood of translation of efficacy observed in those models to clinical benefit. Finally, studies in rodents deliver robust, reproducible data and so it is often unnecessary to evaluate efficacy of new compounds in higher species.

The project uses techniques that can also be used in patients during clinical trials such as detection of sleep-wake cycles *via* electroencephalography and muscle activity *via* electromyography. These techniques will provide efficacy data on key symptoms that will be directly translatable to the clinical situation.

In addition to tolerability studies, pilot studies may be conducted in a small number of animals in order to refine the parameters and methodology for ensuing studies. These are intended to define the risk/benefit ratio of each procedure to generate statistically significant data whilst causing the least adverse effects on the animals.

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 assessment of efficacy in an intact mammalian system is very important in selecting new successful strategies to treat narcolepsy. These development candidates should be highly effective with fewer side effects than current therapies resulting in a reduction in daytime sleepiness and cataplexy and improvements in quality of life of patients.

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

Mice and rats (10,500 and 3000 respectively) These are the maximum numbers to be used over 5 years

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

The majority of studies described in this Licence are well-tolerated by rodents. Mice and rats have been chosen because the sleep patterns and mechanism of sleep disturbances have been well characterised and documented in these animals. It is not expected that serious adverse effects will occur but any side effects are likely to involve body weight loss and deterioration in clinical signs. Any animals exhibiting such signs will be removed humanely from the study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Narcoleptic animal models are required to assess the effect of a test compound on excessive daytime sleepiness and cataplexy (efficacy).

Cell and *ex-vivo* assays can give a good indication of the potential ability of a compound to modulate neuronal activity but they cannot reliably predict *in vivo* efficacy on complex neurological processes such as sleep/wake cycles. *In vivo* models are therefore an absolute necessity to relate *in vitro* data to efficacy in order to predict a potential clinical benefit. In addition, the PK/PD relationship, driven by distribution, metabolism and elimination, cannot be accurately modelled *in vitro*.

Finally, proven *in vivo* efficacy data is a prerequisite of the regulatory bodies who have the authority to approve or reject a new drug application.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Protocols covered by this project licence application are designed to use the minimum number of animals possible.

Tolerability studies are performed with small groups of animals in order to establish the maximum tolerated dose and suitability of dosing regimen prior to larger efficacy studies. Only then, can the more complex *in vivo* efficacy studies commence in the knowledge that the animals are likely to tolerate the compound.

Minimum group sizes for efficacy studies will be calculated using power analysis and will incorporate consultation with a statistician.

The use of techniques that allow repetitive recordings of neuronal and muscle activity and brain neurochemistry will avoid unnecessary terminations and enhance the amount of mechanistic data obtained in a single animal therefore decreasing the number of animals necessary for each particular study.

Refinement

Explain the choice of animals 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.

Refinement

It is well documented that orexin-deficient mice express narcoleptic episodes, which mirror most of the main symptoms of human narcolepsy. These models are based on the same mechanism that has been shown to cause both cataplexy and excessive daytime sleepiness in narcoleptic patients therefore improving the likelihood of translation of efficacy observed in those models to clinical benefit. Finally, studies in rodents deliver robust, reproducible data and so it is often unnecessary to evaluate efficacy of new compounds in higher species.

The project uses techniques that can also be used in patients during clinical trials such as detection of sleep-wake cycles *via* EEG and muscle activity *via* EMG. These techniques will provide efficacy data on key symptoms but also key information on the mechanism of action of the compound tested that will be directly translatable to the clinical situation.

In addition to tolerability studies, pilot studies may be conducted in a small number of animals in order to refine the parameters and methodology for ensuing studies. These are intended to define the risk/benefit ratio of each procedure to generate statistically significant data whilst causing the least adverse effects on the animals.

PROJECT 184

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Modulating immune-cell activation for the treatment of disease.
Key Words	Immunotherapy, Cancer, Immunology, Antibody, T lymphocyte
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Despite significant breakthroughs in the diagnosis and treatment of cancer, cancers remain responsible for the majority of adult deaths, far outstripping the mortality caused by heart disease. Surgery and chemotherapy approaches can reduce tumour size but patients often relapse due to the survival of small numbers of tumour cells. Utilising the patients' own immune system to seek and destroy remaining cancer cells is an attractive adjunct to current treatments (termed immunotherapy) as this can potentially be performed with maximal specificity and minimal toxicity.

In the last 7-8 years, immunotherapeutic approaches have gained increasing attention with clinical trials reporting significantly improved survival in patients with melanoma and prostate cancer. These findings confirmed the feasibility of immunotherapy as an approach for cancer. Nonetheless, the T cells which these therapies activate can be inhibited by signals coming from the tumour cells, or associated tissues. Significant progress has been made in countering these negative signals and new immunotherapy drugs to block these have been game-changers in the field of cancer treatment with impressive responses in hard-to-treat tumour types. Nonetheless, these drugs (e.g. ipilimumab and nivolumab) are associated with significant toxicity and not all patients respond. Broadly our aims are to understand the mechanisms that lead to effective immune cell activation, and to identify novel reagents/treatment approaches to counter cancer growth.

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)?

Immunotherapy has the potential to provide long-lasting protection from tumour relapse in patients. The principles obtained from our work will also inform the fields

of clinical infection, autoimmunity, transplantation and allergy as well as veterinary science.

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

Most experiments will be short lived (in the order of several weeks); very few will exceed 100 days in duration. Animals will be used between May 2017 and April 2022. We anticipate using around 15725 mice during these 5 years.

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

The vast majority of the experiments will result in no adverse effects. When using tumour models, mice will be culled at the humane endpoint. In some cases the administration of immunomodulatory substances may cause transient adverse effects, but these will remain within the moderate severity limit, otherwise the mice will be culled.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We are committed to replacing mice where possible and we evaluate immunotherapeutic agents on cell lines in vitro when we can. However, immune activation influences multiple cell types across the body concurrently and this cannot be adequately modelled in vitro at the current time. Similarly, to study the interactions between an ongoing immune response and a growing tumour, or to evaluate immune-mediated pathology there is unfortunately no viable alternative to in vivo modelling using animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Mice used across experiments are inbred thereby minimising intra-group variability and allowing reduced mouse numbers for experiments. Experiments are always designed with the fewest animals consistent with obtaining statistically valid results. We have performed Power analysis to determine the numbers of mice required to deliver statistically significant results, although through experience we find we can often use smaller numbers of animals without sacrificing statistical significance.

Where appropriate, small pilot experiments are carried out where simple factors such as dose or route of administration are not clear. Where multiple inter-relating parameters are to be evaluated, larger factorial experiments are performed to prevent use of excess mice as controls. In recent years significant technological advances have enabled more information to be obtained from one individual mouse than was previously possible (e.g. using multi-parameter flow cytometry and micro-array technology), enabling multiple parameters to be assessed simultaneously from small samples. These technologies thereby facilitate longitudinal studies and reduce the need to cull multiple mice at different time points to sample from the spleen for instance; we aim to fully exploit these new techniques fully where possible. Tumour cells will be stored frozen when possible to prevent mice being used to passage tumour in vivo.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the least sentient mammal species with an immune system similar to humans. Mice represent a relevant animal model for these studies and the clinical successes now being reported using immunomodulatory drugs against cancer were dependent on data arising from such murine studies. Numerous mouse cancers have been studied and the availability of genetically altered strains, and commercially available reagents aids this research. Environmental enrichment, good husbandry and frequent monitoring ensure high welfare standards.

Few adverse effects are anticipated but, should any occur, rapid steps will be taken to ameliorate them or humanely cull affected animals. All animals are maintained by qualified and experienced animal technicians who are familiar with the models. Any animals which are anticipated to be nearing a defined end-point, or for which a defined end-point is not yet established, are monitored more closely. Should a technician find an animal that has reached an end-point the animal is either immediately culled or the PIL holder is informed that the animal is required to be culled immediately.

Death is not an acceptable end-point for cancer models: we have established end-points for humane culling before pain/distress occurs, based on accepted guidelines. Many tumour lines develop as subcutaneous nodules, allowing easy monitoring of tumour size. However, the visible or palpable size of the tumour is only one of the criteria used for determination of humane endpoint. Experiments will therefore be terminated before tumour size limits behaviours (feeding, drinking, movement) or before or at the first signs of, tumour associated symptoms or poor

condition of the animal according to well defined guidelines (e.g. facial expression scales; www.nc3rs.org.uk/assessment-pain-using-facial-expressions-laboratory-mice-rats-rabbits-and-macaques). Occasionally, following therapy a subcutaneous tumour resolves from the inside out giving the appearance of ulceration; we have adopted a scoring system from Lloyd and Wolfensohn in the Handbook or Laboratory Animal Welfare and Management to ensure that these are managed with minimum adverse effects to the mice. While the maximum severity limit for much of the work to be conducted under this PPL is set as 'moderate', through experience and good management of the mice, we have found under our existing PPL that the actual severity of most experiments is 'mild'.

PROJECT 185

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Regulation of normal and malignant haematopoiesis
Key Words	Haematopoiesis, Leukaemogenesis, Leukaemia Stem cells, Lineage specification
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Leukaemia (blood cancer) is the 12th most common cancer in the UK (2014). There were around 9,500 new cases of leukaemia in the UK in 2014, that's 26 cases diagnosed every day. Incidence rates for leukaemia are projected to rise by 5% in the UK between 2014 and 2035, to 19 cases per 100,000 people by 2035. It is projected that 13,758 cases of leukaemia (8,714 in males, 5,044 in females) will be diagnosed in the UK in 2035. (Statistics from CRUK). The most frequent leukaemia (Acute Myeloid Leukaemia or AML) represents ~ 1/3 of all leukaemia cases. The five-year survival rate is ~ 20%. In patients younger than age 60, ~ 70 percent go into remission after conventional treatment. Those older than age 60 do not typically respond to treatment as well. They also have a higher rate of dying from conventional treatments. There is therefore a clear need for improving survival rates through development of new types of therapeutic agents.

This project aims at defining the programmes of regulation and the mechanisms of action of important genes and their products (proteins) involved in blood cell production (haemopoiesis) as well as to characterise the heterogeneous populations of leukaemia (blood cancer) propagating stem cells to improve our basic understanding of this disease. The molecular mechanisms controlling production, survival, self-renewal and differentiation of normal and cancer stem cells are not fully understood. In the course of the study, mice will be irradiated and bone marrow reconstituted with normal and malignant cells. This approach will allow the investigation of the mechanisms underlying normal as well as malignant (leukaemogenic) haemopoiesis. ?A better understanding of the causes of leukaemia will help identify novel therapeutic targets for treatment of blood malignancies and other cancers in general.

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 results of the proposed experiments will lead to a better understanding of (1) how genes involved in the production of blood function and, therefore, how blood stems cells (HSCs) are produced and maintained and (2) how leukaemia is initiated and propagated. This will have important benefits: (1) The ability to maintain, expand or direct the differentiation of HSCs will impact on both the efficacy and application range of transplantation regimes. This will contribute to improve stem cell therapy through collaboration with local clinicians. In the longer term, new insight into blood stem cell development will help design protocols supporting the generation of these cells in vitro from patient-specific pluripotent stem cells. This would benefit many haematology cell-based therapies; as an example, patients with bone marrow failure syndrome, currently treated with allo-HSC transplantation (with up to 40% mortality rate due to graft versus host disease) would considerably benefit from auto-HSC transplantation as this would increase of the survival rate currently achieved through allo-transplantation. Moreover, this would bypass the problems due to lack of suitable donors. (2) This knowledge will specifically lead to the identification of drug targets and the development of a range of therapeutic opportunities. In the medium term, this will also allow to track leukaemic stem cells (LSCs) in patients undergoing treatment and to determine if current therapies can eradicate LSCs in all patients and if LSC eradication correlates with cure. In the long term, our studies will help identify common markers on LSCs and help develop therapeutic antibodies. These will ultimately be tested in clinical trials and therefore be of interest to pharmaceutical companies and benefit patients with a range of life-threatening leukaemias.

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

We propose to use ~23,000 mice over the next 5 years and ~160 frogs.

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?

Around 40% of mice will undergo mild procedures (conventional breeding and maintenance methods) and will not suffer any harm. Around 60% will be subject to more involved procedures. These mice will typically experience irradiation (to provide “space” in the bone marrow and allow engraftment of injected cells) followed by tail vein injection of blood cells (mouse or human, normal or leukaemic), administration of substances by oral route (to induce changes in gene expression or explore the impact of specific agents/drugs on haematopoietic cells in vivo), bone marrow sampling or peripheral blood sampling two times (to investigate mechanisms of normal blood or leukaemia development). Potential adverse events primarily relate to irradiation and to the nature of the cells injected, resulting in: • Infection due to immune suppression, bleeding due to reduction of the number of blood cells, or diarrhoea and dehydration. • Clinical signs of leukaemia and anaemia, i.e. strong

palour in feet and ears with reduced mobility and/or loss of appetite. We do not anticipate mortality or significant morbidity in any of the protocols at a frequency >5%. Welfare of animals at risk will be carefully and regularly checked. If some animals are in pain or exhibit other adverse effects, pain-killers or other treatments may be given under veterinary direction or humanely killed. Mouse strains showing any unexpected ill-health will be humanely killed. Most animals will be killed at the end of the study through a regulated procedure providing the most humane death possible. In order to analyse the earliest stages of hematopoietic development, we will also use frog embryos. Indeed, very early signals are not easily studied in mammalian embryos in utero and the number of embryos available for study is limited. In contrast, large numbers of frog embryos are freely accessible from the fertilised zygote onwards. Furthermore, developmental mechanisms are highly conserved in evolution so that the frog model can be translated to the human. After collection of fertilized embryos, which is a mild procedure, various reagents are introduced into developing embryos to control gene activity. Embryos will be only allowed to develop to pre-free feeding stages, a non-regulated procedure. There is therefore no adverse effects to be reported.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In vitro experiments using cell lines or primary cells only provide information at one given stage of development/ differentiation, outside a physiological context and with all the artefacts that the use of cell lines can generate. It has long been recognised that the cellular content of a specific cell varies during development, that the microenvironment can influence the biology of a cell and that the whole physiological setting of an animal can modulate biological systems at a molecular level, with obvious implications on gene expression/function. These variations and differences, that cannot be investigated *in vitro,* are to be taken into account to fully comprehend gene regulation and function. This will be studied using mouse and frog models. Moreover, transplantation of human normal and/or malignant blood cells into mice is currently the “gold standard” method to assess human blood cell function in an in vivo context in a whole organism.Therefore, in order to study the full effects of genes that play an important role in humans and their disease-associated mutants, there is no substitute for a mammalian model.

When appropriate, we use a lower vertebrate model, such as Xenopus, to study specific aspects of blood development that do not require transplantation assays.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Transplantation experiments - We are constantly following the field for advances in the generation of more efficient transplantation models. We always perform pilot experiments to establish the optimal number of mice to be used in each experiment.

Statistics - Experiments are always designed to ensure that the minimum number of animals is used to provide statistically significant data. Good experimental design and collaborations within the group aid reduction of animals by decreasing duplicate analyses.

Creation of genetically modified mice - Once chosen, the genes of interest are analysed using a variety of bioinformatics approaches and their expression and function are first studied in vitro, using primary haemopoietic cells and cell lines. Modifications are frequently made in mouse embryonic stem cells and their effects are first tested by experimental differentiation of these cells in culture. The decision to create genetically modified mice would only be taken if the benefit was to be justified in light of these preliminary experiments.

Novel transplantation models: the use of humanized mouse models (growth of humanized ossicles and implantation of scaffolds to recreate a human bone marrow niche) will provide more efficient engraftment of human samples, therefore significantly reducing the number of mice used for xenotransplantation experiments.

By using techniques that have been standardised over the last 30 years and by cryopreserving lines as soon as possible, the overall numbers of animals used will be kept to a minimum

Refinement

Explain the choice of animals 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.

Refinement

Our experimental model uses the mouse as this is a vertebrate species with similar physiological characteristics to humans and adequate for transplantation experiments to test mouse and human blood stem cell activity. Genome sequence analysis has shown a high degree of genetic homology and in both species the number and positions of genes have been well mapped. This is particularly important if one wants to ultimately apply basic knowledge to clinically orientated research.

We also use the frog model because their embryos are available in large numbers, develop externally, and are easy to manipulate. Moreover, a large part of genetic

circuitry controlling blood and vascular development is conserved with mammals (humans).

Measures to minimise welfare costs to the animals:

In the course of these studies, we will introduce two novel xenotransplantation models and a humanised ossicle system. We will also implant biomaterial scaffolds mimicking the extra-cellular matrix to improve HSC niche formation. These models will allow human engraftment significantly faster and more efficiently and therefore will decrease the duration of the experiments.

- Invasive procedures are performed under general inhalation anaesthetic, and the animals are closely monitored after the procedure to ensure that normal function and behaviour is restored. Additional pain relief is given if appropriate. Antibiotics may be given to control any adverse effects of immuno-suppression.
- The lowest dose of irradiation needed for engraftment is typically used.
- To reduce risk of radiation-associated toxicity, mice typically receive radiation as split doses
- We always use aseptic surgical techniques for injections
- Mice which are engrafted with leukaemia are closely monitored so that they are killed at the humane endpoint to ensure that suffering and distress is minimised.
- In general, all animals will be closely monitored during the experimental procedures and up to a few days after when required and any animal showing signs of distress will be humanely killed.
- Regulated procedures are always carried out by appropriately trained personnel.
-
    - All investigators are highly trained and competent to perform the techniques in the animals.
    - The protocols are continually refined to cause the least suffering.
    - Animals are monitored daily and more frequently throughout experiments for signs of distress or disease.
    - Any animals showing signs of distress are isolated and treated appropriately or where required the advice of the vet is sought.
    - Where necessary we have established a detailed set of humane endpoints.

PROJECT 186

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanisms of Heart Failure
Key Words	Heart failure, hypertrophy, hypertension, myocardial infarction, inflammation
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project will investigate the changes that occur within the cardiovascular system during the development and progression of heart failure with underlying disease such as diabetes in order to identify new therapeutic targets and treatments.

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

Chronic heart failure (CHF) is the leading cause for death in the UK and affects up to 2% of adults. It is caused by all heart diseases that increase cardiac workload, such as hypertension, valve diseases and myocardial infarction. Diabetes is also a risk factor for development of CHF and about 19% of CHF patients have diabetes. Many drugs and devices have been developed, but CHF carries an unacceptably high morbidity and mortality. A better knowledge of the mechanisms that underlie the development and progression of CHF is essential to develop new therapies to treat patients with CHF. This work should substantially increase our understanding of the cardiovascular changes which are critical in the development and progression of CHF. By elucidating underlying mechanisms and by undertaking initial experimental studies in animals, this research may provide the basis for devising novel strategies and medicines for treating cardiac disease. In the long term this will benefit patients stricken with this serious and common disease.

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

Mice will be used to model CHF. We estimated, based experience and experimental design tools that up to 31,050 mice will be used in 5 years.

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

Three complementary models will be used which mimic the main causes of human CHF. Surgical models involve constriction of the aorta (a major body blood vessel) or occlusion of left coronary artery which supplies blood to the heart muscle (myocardial infarction). Alternatively, CHF may be induced chemically, e.g. by infusion of agents that increase blood pressure. In some studies, diabetes will be induced either by altered diet or by injection of agents that decrease insulin production. All surgeries will be performed under aseptic conditions and animals treated with appropriate anaesthetics and analgesia. Since CHF develops slowly over time, the animals need to be followed for several weeks. The development of heart failure may be associated with loss of weight, listlessness and rapid breathing. Animals will be closely and regularly monitored during the study. Any clinical problems will be dealt with in consultation with the veterinary surgeon, and where necessary in cases of distress, animals will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Because CHF is a complex disorder involving the interactions of several body systems including cardiovascular vascular, neuronal, respiratory, and endocrine systems, it is not possible to simulate these biological systems completely in a test tube. Thus, there is no feasible alternative to the use of animal models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

All efforts will be made to restrict animal numbers to a minimum and respect the 3Rs. Our breeding programmes are monitored on a regular basis in order to keep numbers to a minimum. For many studies, we will use non-invasive techniques that enable serial assessment of cardiac function during CHF development, allowing reduction in numbers. This is especially valuable when assessing the impact of medicines aimed at preventing or slowing disease progression. We will follow principles of good experimental design to ensure clear answers to questions being addressed while using the minimum number of animals. The proposed methods, experimental design and methods of analysis of the results are based on careful consideration of statistics, mathematics and experience and have undergone

stringent review as part of the grant-awarding process. Wherever possible, detailed studies of cellular mechanisms will be conducted in cultured cells.

Refinement

Explain the choice of animals 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.

Refinement

The study will be performed using mice because all relevant methods and techniques are successfully established in this species, and because of the availability of genetic alterations in mice in particular. These help us to study specific biochemical pathways with a view to understanding disease progression and interfering with it to provide new treatments. The animals will be housed in our modern facility with environmental enrichment, designed to allow the animals a wide range of natural behaviours and some privacy. The husbandry of the animals is according to best practice, involving highly trained staff and is under regular review by our institution.

All surgical procedures will be conducted under aseptic conditions, with appropriate pain relief the highest levels of skilled care after surgery and appropriate veterinary consultation. As the major side-effects occurs within the first 24 hours after operation, animals will be closely monitored at frequent intervals during this period. Careful attention will be paid to heating, analgesia, body weight, surgical wounds-sites, hydration, and signs of pain or distress. During the chronic course of progression to CHF, animals will continue to be carefully monitored and any that are in a poor clinical condition will be treated in consultation with the veterinary doctors, and humanely killed if appropriate.

PROJECT 187

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Raptor health in Scotland
Key Words	Conservation, Ecosystem, Indicator, Raptor
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
Yes	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The objectives of this project are to study and improve our understanding of Scottish raptor health and the effects the environment could be having on it.

Raptors, as predators, at the top of the food chain are good indicators of the health of an ecosystem. Bioaccumulation of potentially toxic chemicals can occur at each level of the food chain and be at their highest level in predators. Decrease in population numbers or disease in prey species at lower levels of the food chain can quickly affect predator populations.

Some Scottish raptor populations are recovering from threateningly low numbers, but their recovery is still facing difficulties. The relatively high numbers of adults, but low number of juvenile or sub-adults, and low productivity of some raptor territories due to early deaths in the nest are a few of the ongoing threats to the recovery of these species. Persecution is a well-known threat hampering species recovery, but is not the only one. Early deaths of young in the nest and poor productivity of territories in certain areas of Scotland cannot be wholly explained just by a lack of food resources and/ or persecution. It is necessary to identify and understand the environmental and disease threats to raptor species, and to investigate the causes of early chick deaths and low productivity in raptor territories in some areas of Scotland.

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 this project are to the health, welfare and conservation of UK raptor populations. By gaining an understanding of the effects that the environment may be having on raptors we can begin to understand the impacts that human practices in the environment could be exerting on these species, other animals and humans. This project will work specifically towards Scottish management plans for

raptor conservation and management advice for the different locations in collaboration with local bodies as appropriate.

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

Up to 180 raptors over 4 years

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

We will be studying free-living raptors by sampling them whilst at the nest prior to fledging. We will work side by side with raptor experts who routinely handle them under ringing licences at this time of the year to mark them. We will take blood samples and cloacal swabs before they are returned back into their nests. The severity of the category of these procedure is mild. Expected adverse effects of this are negligible to very low (<1%). We are using standard blood sampling and cloacal swabbing techniques for avian species including wild raptors. If any of the individuals are found to be distressed as a result of the blood sampling or cloacal swabbing they will be given time and space to relax before reattempting, if unsuccessful they will be returned to their nests without procedures being performed. All chicks will be returned to their nest as soon as possible at the end of the procedure. Previous experience of raptor workers worldwide and the applicant has shown that there is no risk of rejection or abandonment of chicks by parent birds after interventions from humans, including handling, ringing and blood sampling and that these procedures are tolerated very well in raptor species, with resumption of normal activity (as monitored by CCTV) almost immediately after return to the nest.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The purpose of this project is to study the health of Scottish raptors and the effects the environment could be having on them. Therefore free-living raptors need to be examined and sampled in order to identify any health problems they may be having. Alternatives to using wild raptors are not possible if the objectives are to be achieved

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

In the first year we aim to sample as many raptor nestlings as possible from different areas in Scotland. This will give us the best opportunity to see what effects the environment is having on raptor populations in the different locations. After analysis of first results we will re-evaluate our sample size based on prevalence of the different pathogens, heavy metals, other toxicants or any other disease conditions identified.

Refinement

Explain the choice of animals 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.

Refinement

Raptors in Scotland are at the top of the food chain and can be very sensitive to environmental practices. Some Scottish raptor populations have been slowly recovering from the brink of extinction but full recovery is still facing various threats. This project endeavours to understand what threats are affecting raptor population recovery and early life stages losses of individuals in certain territories.

The catching and handling techniques have been used by raptor experts and blood sampling and cloacal swabbing techniques are widely used for avian species and all techniques used by this project will be performed by trained and experienced personnel.

Intervention time for blood sampling and cloacal swabbing will not exceed 10 min per individual and when all individuals from the same nest have been processed and assessed as fit for release,they will be returned to the nest. Access to the nest depends on their nature and location. Climbing preparation for ascent or descent to the nest, followed by access to it, retrieval, ringing and measuring of individuals can take on average 30 minutes.

PROJECT 188

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Foetal origins of bone formation
Key Words	maternal Diet, Bone, Offspring, Vitamin K-dependant proteins, Vitamin-K
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To assess the role of the mother's diet during pregnancy on subsequent bone development and growth in the offspring. Briefly, the mother's diet during pregnancy has been found to be key to the likelihood of her offspring developing heart disease, obesity, or diabetes in later life. Evidence suggests osteoporosis may be an additional risk to these offspring. We want to know how important the mother's pregnancy diet is on long-term growth of bones in her offspring.

The mother's diet during pregnancy has been found to be key to the likelihood of her offspring developing heart disease, obesity, or diabetes in later life. Evidence suggests osteoporosis may be an additional risk to these offspring. We want to know how important the mother's pregnancy diet is on long-term growth of bones in her offspring.

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 study will determine the extent to which maternal diet (and postnatal diets) can affect normal bone development and growth in the offspring. This is important, as we need to know the long-term consequences of the current unhealthy diets in the Western world.

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

We estimate 300 rats and 950 mice will be used over a 5-year period in this study. These species have been used for dietary studies for many years. We must use the same species so we can use results from previous studies to add to the results found in this study. The animal numbers used are the minimum required to obtain

meaningful results. These numbers are checked by funding organisations to confirm this is the case.

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

The rodents may receive a diet that causes them to become obese. Long term feeding of these unhealthy diets may also cause heart disease, raised blood pressure, diabetes, as they do in humans. In order to x-ray animals, it will be necessary to anaesthetise them. However, as with any animal, there may be an adverse reaction to the anaesthetic. The likely/expected levels of severity will be mild, and at the end of the experiments, the animals will be terminated by appropriate schedule 1 method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We propose to determine how the maternal diet can affect bone development and growth in the offspring. Pregnancy is a highly complex system involving precise timings, hormones, specific cell types, genetics and nutrition which cannot be modelled without using animals. How maternal nutrition and pregnancy affect bone development and growth in the offspring is not known. While it may be possible to grow calcified tissue on collagen structures from blood-derived stem cells in the laboratory, these “bones” are in isolation from other organs and hormones. Hence, maternal nutrition may affect a multitude of hormones in different ways, altering not only hormone levels, but organ growth, and so-called epigenetic factors which could, in turn, affect the stem cells and growth of subsequent generations. In order to be able to produce an *in vitro* model system, we must first know if our targets of choice are altered in model animal systems.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

[REDACTED]

By using an *in vivo* micro-CT system we will be able to analyse bone development in the same animal over its lifetime, thereby increasing sensitivity and, critically, reducing animal numbers

Refinement

Explain the choice of animals 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.

Refinement

The mouse models will include C57BL/6 mice which have been widely used for high-fat nutritional studies. In addition, the MGP knockout and other genetically altered models are also based on this strain.

Rats have been used for nutritional studies for over 10 years, and Wistar rats remain a widely accepted model.

Minimising suffering

The research procedures in the project will not exceed the MILD severity level. To minimise suffering, all animals will be assessed daily for signs of distress or ill health. Severe malnutrition will not be used in this project. Any animals exhibiting a reduction in weight gain of 10 % body weight over a 3 day period, or showing signs of distress and/or pain will be killed by a Schedule 1 method. Handling will be minimised to routine husbandry and procedures required for the project.

PROJECT 189

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Metabolic functions of insulin signalling and resistance in the brain
Key Words	Insulin resistance, brain, metabolism
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To understand how the central nervous system senses insulin to regulate metabolic behaviour

To understand how insulin resistance in the brain affects metabolic functions

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 aim of this study is to investigate brain regulation of glucose metabolism and feeding behaviour. We will look at the effect that the insulin resistance in the brain has on whole-body metabolism. This work will advance fundamental scientific knowledge in the central nervous system (CNS) control of metabolic behaviour. The focus will be to understand how central insulin resistance occurs in order to find approaches to restore insulin sensitivity in the CNS for improving whole-body metabolism. The brain senses insulin levels to regulate glucose metabolism and feeding behaviour. Unfortunately, the brain can also develop insulin resistance, and consequently lose its ability to regulate metabolic behaviour. Restoration of the brain`s ability to modulate peripheral metabolic functions could be a good approach to fight the development of obesity and type 2 diabetes. The proposed work aims to understand how insulin resistance in the brain is developed during obesity, with particular focus on the effect that altered mitochondrial functionality has in the development of insulin resistance (mitochondria produce the energy of the cell and regulate cellular metabolism). Considering that mitochondria are inherited, the repercussions of having altered mitochondrial functionality can also be transferred to children by affected parents.

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

We expect to use 1124 adult male rats over the 5 year duration of the project.

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

The majority of the procedures will require recovery from anesthesia following surgical procedures. The surgical procedures will involve the insertion of cannulas in specific areas of the brain for the purpose of infusing substances and the insertion of catheters in the blood vessels in order to infuse substances and withdrawal of blood. Brain cannulas are inserted using a specialized equipment. We have extensive experience in performing such surgical procedures and do not envisage adverse effects during post-surgical period. Post-operative pain relief is routinely given to all animals and pain response is monitored on a score sheet. After surgery, the rats will be housed individually to avoid the dislodging of the catheters. We will use transparent cages near each other so the rats do not feel isolated. At the end of experiments animals will be humanely killed to allow for tissue collection.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Since this study focuses on understanding the physiological impact of the development of insulin resistance in the brain, there is no possibility to substitute the use of animals with alternative systems. We will study the effect that insulin resistance in the brain has in the regulation of glucose metabolism and feeding behaviour.

Rodents, especially rats, are the best animals to use to perform metabolic studies. In particular, their glucose levels are very stable and in addition, the rodents have a fast and complete recovery after brain surgery and also recover well after vascular surgery.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used will be minimised in several ways. Tissues from the same animal will be used for different objectives where possible. This reduces the

total number of animals that would otherwise be required if a single animal was used for each procedure. In addition, each experiment is designed to maximise the amount of information gleaned since one experiment often combines different approaches to verify the information.

Refinement

Explain the choice of animals 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.

Refinement

The experimental procedures require the use of rats because they have the best system to enable us to perform metabolic studies. Due to their anatomy, is easy to perform brain surgery in rats and they have a complete recovery after surgery. All our preliminary data has been obtained in rats and previous studies have been performed with rats.

All the surgical procedures will be carried out under aseptic conditions. After surgery, we will constantly monitor the rodents until recovery and we will apply post-operative analgesia until the animals are fully recovered.

PROJECT 190

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Control of Biological Medicines and Vaccines
Key Words	Quality, Control, Biological medicines
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

In order to ensure that biological medicines are both safe and effective it is necessary to thoroughly evaluate their quality and activity. Biological medicines are very complex materials that include; vaccines; products derived from human blood such as clotting factors and immunoglobulin; hormone drugs such as Erythropoietin and Human Chorionic Gonadotrophin; and an increasing number of new 'high-tech' biological medicines such as therapeutic monoclonal antibodies for treatment of cancers and immune disorders produced by the latest techniques of genetics and molecular biology. For many biological medicines it is a legal requirement that each batch is examined and approved before release onto the market. Such 'control' or 'batch release' testing involves laboratory tests on the product itself and investigation of adverse responses to new biological medicines. Animals are used for control testing where it is prescribed in the European Pharmacopoeia or in product licences as a legal requirement. Animals are also used to develop, validate and produce reagents for alternative assays that are proposed to European Pharmacopoeia and product licensing bodies. Where validated alternative methods are available then the maximum use of such procedures is made to minimise animal testing.

What are the potential benefits likely to de

rive from this project (how science could be advanced or humans or animals could benefit from the project)?

Science will benefit from this project through a greater understanding of the mechanisms of adverse drug responses to new biological medicines. People will benefit from this project through the use of proven safe and effective biological medicines. Animals will benefit from this project through the development and validation of safe alternative assays that do not require animal testing.

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

The number of animals required per dose and numbers of groups are specified in regulatory documents and in-house guidelines which consider the minimum required to give reliable results of satisfactory precision determined by statisticians. On averages we expect to use per year approximately 1300 rodents, 2 sheep, 3 alpacas and 2 guinea-pigs and 1 non-human primate based on previous usage.

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

The procedures employed are minimally invasive involving administration of small volumes of substances by injection or withdrawal of blood involving the least possible suffering. The procedures utilised involve administration of human biological medicines by the intravenous, subcutaneous or the intraperitoneal route, withdrawal of blood from superficial vessels and administration of immunogen and adjuvant by the subcutaneous or intramuscular route. The licence contains 4 protocols classified as mild and 4 classified as moderate. Typically the majority of animals used in protocols are not expected to experience more than transient discomfort. All animals are frequently inspected by experienced personnel and/or a veterinary surgeon. Possible adverse effects include haematoma at the site of blood withdrawal, local irritation or abscess formation at the site if injection that will be treated with antibiotics under veterinary direction, or mild anaemia with Erythropoietin assays. Any animals showing signs of adverse effects will be appropriately managed either treated under veterinary direction or humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animal species are employed on the basis of similarity of their endocrine or immune systems with humans and adequate protein sequence homology, where no validated alternatives exist or where they are prescribed in the European Pharmacopoeia and in product licences.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The protocols employed have been refined by collaborative studies involving significant statistical input over the years and are modified and upgraded on an ongoing basis by our statisticians. The number of animals required per dose (group) and numbers of groups are specified in regulatory documents, relevant European Pharmacopoeia monographs and in-house SOPs or are considered the minimum required to give reliable results of satisfactory precision.

Refinement

Explain the choice of animals 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.

Refinement

Animals are housed in a dedicated facility and well cared for by professional animal care staff. Additional measures such as provision of species appropriate enrichments are used. Animals are housed in groups when appropriate and scientifically valid. Score sheets and monitoring regimes are routinely applied.

Most of the protocols are defined in the European Pharmacopoeia and in product licences and so scientists are using the protocols, procedures and models that they are legally required to follow. We will continue to develop alternatives to *in vivo* testing and to validate the alternatives and to propose them to Pharmacopoeias and product licensing bodies.

Since preclinical safety testing in macaques failed to predict an adverse response to TGN1412 at clinical trial, if we can demonstrate that a humanised mouse model would have predicted an adverse response then this will be a powerful argument in favour of its adoption and will encourage a reduction in the use of macaques in preclinical studies. Micro-sampling of blood will be applied when repeated sampling of individual mice is required during the immunotoxicity studies.

PROJECT 191

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Development of therapies for the prevention and treatment of cancer
Key Words	Cancer, Prevention, Phytochemical, Repurposed, Therapy
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Our research aims to identify and develop therapies to prevent cancer and we use our laboratory results to design and conduct clinical trials. We focus on existing well-established drugs or diet-derived compounds that have good safety profiles so they can ultimately be taken by healthy people on a long-term basis. We adopt an array of *in vitro/ex vivo* and *in vivo* tumour models to test selected compounds and identify the best dose to halt tumour formation. To gain further information on how best to implement these compounds for clinical use we also study their ability to accumulate in the target tissue, investigate their mechanisms of action and determine how other dietary, lifestyle or genetic factors influence their effectiveness. We are also interested in combining these different compounds to investigate whether their combinations enhance 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 work conducted under this project will lead to the identification of safe diet-derived agents and repurposed drugs with the potential to prevent cancer in humans and/or improve the treatment of cancer, when used in combination with chemotherapy. The results will be used to optimise the design and monitoring of clinical trials to test the therapies in volunteers and patients. We expect that the information gained will also help us to identify which people are most likely to benefit from taking a particular therapy to prevent cancer so trials can be focused specifically on these people. Ultimately, the successful discovery of effective therapies would help prevent an immense amount of human suffering and save precious health service resources.

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

All our studies are conducted using mice. We estimate that we will need to breed and use about 6,500 mice over 5 years for our research.

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

The adverse effects are most commonly associated with the development of tumours or precursors to tumours in the mice. In the genetic models of colorectal cancer mice develop anaemia and lose weight; we often end our experiments before they develop these symptoms but in longer term studies the mice are most often humanely killed because they lose 20% of their body weight. In a different type of model where mice are injected or transplanted with cancer cells/tissue under the skin, mice are humanely killed because the tumour grows to the limit allowable. We don't normally see any adverse effects associated with the compounds that we give the mice. Some models involve surgical implantation of tumour cells/tissue, most frequently under the skin. Where surgery is required we will minimize wound breakdown and infection using appropriate aseptic technique and suitable training. Pain will be controlled using effective anaesthesia and analgesics.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The use of live animals is essential to assess the effects of promising preventive therapies in terms of efficacy and tolerability/toxicity on all major systems *in vivo.* Experiments in mice are required to take into account the integrated effects of metabolism, distribution and elimination and also to study interactions between diet/metabolic factors and preventive therapies. Before we get to this stage however, we make extensive use of cell lines and state-of-the-art *ex vivo* cultures of intact pieces of tissue and 3-dimensional cell structures, derived from humans and mice to screen agents. We use these models to identify the most active compounds and understand how they work at a basic cellular level. They enable us to conduct dose-response experiments and evaluate combinations so that only the most promsing agents, at their predicted optimal doses, are subsequently advanced to *in vivo* testing in mouse models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

[REDACTED]the minimum numbers of mice are used to generate statistically significant results for each type of study. For example, we have devised a breeding strategy that minimises variation in tumour development in our genetic models of cancer and therefore leads to more consistent results, which minimises animal numbers.

We will work closely with a biostatistician to design experiments and determine appropriate group sizes based on our previous data or pilot studies/*in vitro* data. The results obtained will be subjected to detailed statistical analyses to obtain the maximum amount of information possible from each experiment.

Refinement

Explain the choice of animals 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.

Refinement

Our main focus is the development of cancer preventive strategies and for this reason we are interest in the initial phases of tumour establishment. This is why we have carefully selected genetically modified mouse models that carry alterations in genes involved in the initiation of tumour development. For example, our colorectal model is based on the selective deletion of the Apc gene. Mutations in this gene are known to initiate colorectal carcinogenesis in the vast majority of human cases, therefore this mouse is an ideal proxy to the human situation.

With regard to xenograft and allograft models, they offer alternative advantages. Xenografts have been extensively used to test the efficacy of drugs and compounds on human cells in a “surrogate” *in vivo* model. In the past few years, we have also implemented the most refined xenograft models, based on the surgical implantation of primary cancer tissue in immunocompromised animals. These patients derived xenografts (PDX) remove any potential bias associated with using decades-old laboratory cell lines that have greatly diverged from their tumour of origin. PDXs offer the possibility of testing compound efficacy or to study modulation of tumour biomarkers using material freshly isolated from patients. Xenograft/allograft models are similarly useful when suitable genetically modified mouse model of disease are unavailable.

We have gained extensive experience in the management of these animal models and have learnt to quickly identify adverse effects and spot early signs of distress, so animals are culled before disease progresses, further minimizing suffering. We also strive to avoid as much as possible single housing when randomly allocating animals to study. We have refined our surgical skills and prepared detailed standard

operating procedures (SOPs) for researchers to follow ensuring that animals are properly cared for pre and post-operatively and that appropriate analgesia is provided. We are experienced in dissecting primary samples and identifying which regions of the primary tumours are more likely to successfully graft.

PROJECT 192

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Regulation of brown and beige adipose tissue
Key Words	Adipose tissue, Development, Metabolism
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We aim to improve our understanding of the regulation of adipose tissues, in particular brown adipose tissue function and the process by which white fat can show features of brown fat and thus becomes beige fat (i.e. the 'browning' process).

We will examine whether dietary, environmental and pharmacological stimuli at various stages of development can modulate these processes. This is important as brown (and beige) adipose tissue is a highly promising target tissue in the combatting obesity and/or diabetes due its duel capacity to rapidly generate heat and oxidise large amounts of glucose and fat.

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

The work undertaken in this programme will greatly enhance our knowledge as to how the development and regulation of brown, beige and white adipose tissues are modulated during different stages of development, and with obesity and how we can improve the adipose tissue phenotype through relevant interventions. The results of this work will therefore improve our chances of potential success in treating obesity and related heart disease and diabetes.

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

450 rats over 5 years.

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

The main purpose of this project will be to examine the impact of obesity and a range of interventions designed to either reduce or alleviate its adverse effects on the heart

and glucose regulation. We will achieve this by manipulating brown adipose tissue that has the capacity to use large amounts of glucose and lipids as fuel to generate heat. We will also examine the impact of exposure to stress at birth on its function. The expected level of severity will be moderate as it is not expected that any animal will show clinically related symptoms to obesity given the comparatively short time scale of the proposed studies. The types of interventions to be examined are primarily physiologically based and tailored to the animal's obese phenotype and adverse effects are not anticipated. All animals will be euthanased at the end of each study to enable tissue collection.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

There is no alternative to using animals in this project as we want to correlate in vivo and anatomical changes in brown adipose tissue (BAT) function with a specific environmental challenge that is not possible in humans. Comparable tissue samples cannot be taken from humans (particularly infants and children) given the small amount of BAT they contain and its main anatomical location i.e. supraclavicular region. We will be then able to compare traditional biochemical and molecular measurements of BAT function with those measured in vivo during development. A comparative study in humans would be to examine healthy human subjects using thermal imaging but these types of studies require further validation using the types of in vivo studies we are proposing in this project.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Where dosage or length of an intervention has not been tested previously in the literature we will perform pilot studies to properly inform as to the correct duration, dosage and 'n' per group thus reducing animal numbers. By examining a variety of interventions in one experiment we can gain a more comprehensive understanding of the obesity phenotype and its response to interventions designed to promote BAT function. This is important as there is often wide variability in the response to a given intervention between groups, thus by undertaking each of these in individual groups may not prove useful if final body weights and adiposity are very different.

All studies will be conducted and written up according to ARRIVE guidelines. The design of individual experiments will involve factorial designs where appropriate in order to maximise the information obtained from the minimum resource.

Refinement

Explain the choice of animals 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.

Refinement

Rats are a standard experimental model for BAT development and obesity. They are the gold standard animal model for brown-white adipose tissue studies and the impact of modulating the function of these tissues on obesity. The experiments intended are predicted to cause little distress in the animals as they are primarily based on standard and a highly palatable diet. Both the thermoneutral temperature (26-28°C) and a lower temperature (20-23°C) are commonly used in rats and are within the normal range they would experience in the wild. Swimming as a form of exercise is commonly used in rats that are capable of swimming for hours before fatigue and prior to starting the training protocol rodents will be accustomed to the procedure.

Rodents are also the best model for studying developmental effects as they have an immature neural network at birth. For the whole body vibration (WBV) studies, the range to be used are similar to a range experienced by animals or humans during vehicular transport (i.e. a range of accelerations, rather than a static average).

Social distress to the animals is reduced by group or pair housing them and providing necessary nesting and tubes so they can manipulate their own environment and be able to huddle with companions.

PROJECT 193

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanisms of sleep and absence seizures
Key Words	Epilepsy, Sleep
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

To characterize the mechanism of generation of absence seizures and different stages of sleep. This knowledge is essential to identify novel targets for sleep disorders and childhood and juvenile absence epilepsy.

Animals will be implanted with electrical and imaging devices to allow monitoring and selectively modulating the electrical activity of different populations of brain cells which are important in eliciting absence seizures or the various stages of sleep.

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 short term benefits of the present project are to provide a detailed mechanistic picture of the molecular, cellular and neuronal network processes underlying absence epilepsy and the various stages of natural sleep. The potential long term benefits of the present project include the development of novel pharmacological entities for the treatment of typical and atypical absence seizures as well as different sleep disorders.

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

Mice (8000) and rats (6500) 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 levels of severity? What will happen to the animals at the end?

We do not expect major adverse effects resulting from the procedures that the animals undergo. Some temporary discomfort following the surgery may occur in some animals, but overall the expected maximum level of severity of all the procedures will be mild.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Existing computer-programs that simulate the rhythmic electrical activities observed during absence seizures and different sleep stages are at present highly inadequate in describing these firing patterns and associated membrane potential changes, for the simple reason that they lack the necessary biophysical information on neuronal membrane currents, i.e. the very same knowledge that our project aims to collect.

Absence seizures, non-REM sleep oscillations, EEG rhythms of relaxed wakefulness and electrical activity linked to perception cannot be reproduced in cultures of immortalized human or animal cell-lines.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Whenever possible animal numbers will be minimized by the use of computer programmes that in part simulate neuronal activity during epilepsy and sleep, and by our 30-year experience in the field that ensures that the maximum quantity of data is obtained from each animal.

The use of light activation of neurons ensures that fewer control experiments are required for achieving the stated objectives compared to other mean of neuronal activation.

Refinement

Explain the choice of animals 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.

Refinement

The following approaches ensure our continuous commitment to refinement:

a. we always select the best available drugs, transgenic species and strain, even if this often results in higher purchase and maintenance costs;

b. we never move to the *in vivo* experiments until we have solid *in vitro* data, thus ensuring optimal use of the *in vivo* preparations and strong minimisation of animal suffering;

c. our established collaborations to develop computer models of the electrical activities of single neurons and neuronal networks continues to provide us with the crucial testable hypothesis, thus helping us to refine the experimental procedures towards protocols of lower maximal severity;

d. continuous advice from animal care staff and NVS ensures optimal housing conditions for the chronically implanted animals;

e. high standard of environmental enrichment and play opportunities is always provided before and after the procedures, except in special cases, i.e. only for a few days immediately following any surgical intervention.

PROJECT 194

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Glucocorticoids and stress in the development of diabetes and obesity
Key Words	Diabetes, Stress, Obesity, Steroid Treatment, Developmental Programming
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Stress is known to be a contributory factor in the development of obesity and diabetes, two of the biggest healthcare burdens in the UK at present, costing the NHS £10 billion per annum. To investigate the mechanisms involved in the development of these metabolic side effects, our objectives are four-fold.

1) To understand how glucocorticoids cause obesity and diabetes. Glucocorticoids are stress hormones but synthetic forms can be used in the treatment of a wide range of disorders including asthma and rheumatoid arthritis. The incidence of diabetes is increased 48% in patients with rheumatoid arthritis treated with glucocorticoids for 6 months.

2) To develop treatment regimes to reduce or delay the occurence of obesity and diabetes, using the knowledge gained from objective 1.

3) To examine how chronic stress leads to obesity and diabetes, by understanding both the early and late phases of this process.

4) To investigate the mechanism by which a mother’s stress and/or obesity can lead to obesity and diabetes in her progeny.

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)?

These studies will advance the knowledge of how stress and glucocorticoids can lead to development of diabetes and obesity. By understanding these mechanisms we are hoping be able to develop a dosing regime for glucocorticoids that may reduce some of these side effects. An alternative approach would be to develop a co-therapy (in conjunction with a pharmaceutical company) to reduce the obesity and

diabetes associated with stress and glucocorticoids. The studies are also of direct relevance for veterinary medicine and improved scientific method, as complications associated with chronically increased glucocorticoids levels are common in animals. Furthermore, we will be able to generate results which may influence maternity care to reduce obesity and diabetes in the next generation.

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

Over the 5 years of the project we expect to breed up to 7500 genetically modified mice Over the 5 years of the project we expect to use up to 5000 mice and 2100 rats

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?

Our experiments involve giving the animals cumulative mild stressors leading to an overall moderate experience up to a 4 week period (eg. damp bedding overnight, short periods of immobilisation in a tube (28mm diameter) with appropriate ventilation for up to 30 mins)), altering their diet by feeding them an unhealthy high fat diet or restricting their food, or giving them glucocorticoids usually in their drinking water. These stressors are not expected to cause any lasting harm . Although, given the nature of the experiments, some of the mice may develop diabetes, leading to a moderate level of severity. Some animals will also undergo invasive surgery, where we implant drug delivery devices (in place of daily injections) or inject directly into the area of the brain we are interested in, to place drugs in this region or using technologies that will manipulate the pathways we are interested in. All our experiments will be designed to reduce the level of suffering or harm to the animals. At the end of the study or in the unlikely event that an animal is suffering, they will be killed humanely.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Non-animal alternatives, such as model cell systems, will be utilised for analysis of molecular pathways involved in glucocorticoid actions within brain cells and peripheral metabolic tissues (e.g. liver and adipose). However, the changes that occur in response to altered diet and/or glucocorticoids and stress affect whole-body energy metabolism and need ultimately to be studied in vivo. There is currently no cell system to replace assessment of whole animal’s physiological responses.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

From our previous work we have experience of how to design the experiments to minimise the number of animals used in order to generate meaningful data. Statistical powering will be used to determine the minimum number of animals required to be able to detect the desired size of biological effect as statistically significant. Typical values for significance and power are 5% and 80% respectively. In addition, where such data is available, the power analysis will be updated for a model periodically, as improvements over time may lead to a reduction in the number of animals required. Where needed, we are able to get advice from an experienced statistician to help plan any new experiments in order to get the maximum benefit.

Refinement

Explain the choice of animals 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.

Refinement

Our research projects will only use rodents. We are unable to use zebrafish or other lower organisms, as the models we require (such as diet-induced obesity) are established and validated in rats and mice. We will predominantly be using mice, as the mechanisms we are examining require knock-out models where genes have been manipulated within specific tissues.

We will specifically select acute and chronic stress models which cause the least pain or distress and that have been previously used and validated as being effective. Protocols where we modify diet are all known to have mild effects and any changes in glucose tolerance can be achieved with minimal distress. Part of our rationale is to evaluate stress hormones, and how these change in response to the manipulations used. Therefore we will monitor the stress hormones in many of our studies enabling quantification of the degree of stress in our models.

PROJECT 195

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Cancer development, growth, detection & treatment
Key Words	Tumour, Angiogenesis, Stroma
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Advanced cancer is a global problem that affects millions of people every year. The numbers of people that die of cancer are predicted to increase over the next 20 years unless there are improvements in the early detection and treatment of cancer. Thus there is an urgent need to improve our understanding of multiple aspects of cancer if we are to increase patient survival rates.

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

We believe that this research will inform us better about human cancer and how to detect and treat it efficiently, and, in the future, allow us to test potential new treatments.

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

Over 5 years we will use approximately 60, 000 mice in our breeding procedures and 51, 000 mice in experimental procedures. We use the minimum number of animals required for experiments to be statistically sound.

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

We keep suffering to a minimum by ensuring that staff are familiar with the relevant end points and possible adverse effect. When designing experimental protocols, the animals' welfare and the minimisation of suffering are both at the forefront of design. We include wherever possible, the use of anaesthetics and analgesics and non-invasive techniques. In this project mice will generally undergo procedures that involve primary tumour growth and metastasis and therapeutic intervention. Thus the expected adverse effects will be cancer growth.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacemen

Tumour growth and spread is a complex process involving several cell types and different cellular interactions. This complexity cannot be recapitulated in any *in vitro* system or lower organismal system such as flies or fish. We are not aware of any alternatives to the utilisation of mice for *in vivo* experiments. In parallel with the *in vivo* studies, simple cellular systems will be tested using *in vitro* or *ex vivo* assays and organotypic cultures.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

All experiments will be carried out following good laboratory practice. All experiments will be designed after careful examination of the literature, with regards to treatment ie. drug doasge, treatment regime. Wherever possible use of *in vitro* assays will reduce the numbers of animals required.

Sample size calculations will be performed before each experiment so that experiments are adequately powered statistically, in consultation with our in-house statistician. Many of these are already done for the experiments planned in this proposal. Using the appropriate numbers of animals is essential to answer reliably the questions we are addressing. This ultimately avoids wasting animals and repeating experiments unnecessarily. We will also maximise thee amount of information we obtain from each animal.

Imaging technologies will be a vital tool in reducing the number of animals required for each experiment as this removes the need to kill animals at time points to observe tumour progression.

To minimise confounding factors we will undertake the following:

- **experimental design will be well planned with constant checks**
- within each experiment, animals groups will be the same age, sex and same genetic strain and maintained under the same conditions
- within each experiment, mice will be randomly assigned to different groups
- experiments will always be statistically controlled

When reporting on *in vivo* experiments for publication we will conform to the ARRIVE guidelines to minimise unnecessary studies.
We will consider archiving genetically modified mouse lines as frozen sperm or embryos to reduce the number of living mice we have to maintain and to allow sharing of these lines between researchers, providing further opportunity for reduction.

Refinement

Explain the choice of animals 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.

Refinement

Cancer in mice provides a comprehensive model of human cancer growth and spread. In particular the genetic basis of cancer is excellently represented in genetically modified mouse models of cancer. Also, mice provide us with the opportunity to test the requirement of specific genes during cancer progression by using genetically modified mice. Thus mice provide us with the most refined model of cancer for our study.

All of our mice are kept in IVCs (individually ventilated cages) under barrier conditions, to avoid any infections in immunocompromised mice. Mice are monitored daily for any adverse effects to avoid suffering at all times. We have extensive experience of working with genetically altered mice so we are aware of health problems and can take timely action to minimise suffering, to ensure pain relief is quickly administered whenever necessary and we have clear guidelines on humane endpoints.

To limit the severity in our cancer models we will:

- use the minimum number of cells in the smallest possible volume
- measure tumours regularly to prevent tumours exceeding the legal size limit
- determine humane endpoints to produce valid scientific outcomes
- image mice to detect unexpected sites of tumour development
- use fluorescently labelled cells to image mice for tumour spread and metastasis
- perform pilot studies on orthotopic cancer models to characterise kinetics of tumour growth and metastasis

PROJECT 196

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Joint homeostasis and osteoarthritis
Key Words	Osteoarthritis, cartilage, microRNA, diet, proteinase
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Osteoarthritis (OA) is the most common form of arthritis yet we do not have any treatments that are able to arrest or prevent disease. At the present time treatment is by pain relief and ultimately surgical joint replacement. For some forms of OA such as that affecting the hands, there are no effective therapies at all. Currently, around 8.5 million people in the UK have moderate to severe OA and of these, around 6 million are in constant pain.

OA is characterised by loss of the articular cartilage, the smooth tissue at the ends of bone that allows frictionless movement of the joint. When this is eroded, many changes occur in the joint including remodelling of the bone and inflammation of the lining of the joint. These are thought to give rise to pain and stiffness that is characteristically experienced by the majority of individuals with disease. Despite it being a very common disease, we still have little understanding of the processes and molecules that are important in driving it.

The objectives of this programme are threefold: (i) to understand the role of the proteins which directly break down components of cartilage in the joint in the development of the joint, its ageing and in OA; (ii) to understand recently discovered so-called microRNAs in the joint in the development of the joint, its ageing and in OA; (iii) to ascertain if molecules from the diet prevent or slow the progression of OA

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

Work already published has demonstrated that it is possible to identify new targets for OA from animal models of disease. Some of these are currently being explored by the pharmaceutical industry. Strategies will be broadly divided into two: (i) therapies that prevent disease – this may be most appropriate for individuals who are ‘at risk’ in some way from lifestyle factors e.g. joint injury; (ii) therapies that arrest

or reverse established disease by blocking the pathways that drive on-going disease As OA is a massive burden to society (through days off work due to pain as well as the expense of performing joint replacement surgery), any improvements in the treatment of this condition will be of high relevance and substantial benefit. .

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

#Mice including genetically modified. Approximately 4,850 mice will be used 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 levels of severity? What will happen to the animals at the end?

Genetically altered animals are bred and genotyped by taking samples using ear notches. These animals and normal (wild type) animals then undergo procedures such as ageing, surgery for the induction of OA (as below), the administration of substances via injection or gavage, imaging (under anaesthesia) and blood sampling. OA is induced in mice by a small surgical operation under general anaesthetic. This causes instability of the joint and is akin to the type of injury sustained by a footballer or skier following a twisting accident (“torn cartilage”). The mice tolerate this procedure well and despite having some pain immediately following surgery (lasting about 5 days) are fully active and apparently pain free up until about 8-10 weeks following the operation. At this stage only do they experience pain. On both these occasions, analgesics are used. Most experiments are terminated at 8 weeks after surgery but some animals may be kept for up to 12 weeks. At the end of the experiment, animals are killed. Adverse effects due to the administration of substances, blood sampling and imaging are unlikely. Both surgery and the adminstration of substances is carried out according to the published guidelines and principles of LASA (Laboratory Animal Science Association).

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

OA is a challenging disease to study since it progresses very slowly, can be different in different patients and is not reported to doctors until it is already in its later stages. Getting clinical samples is difficult and the only available tissue comes from joint replacement surgery at the very end stage of the disease.

Our lab has always used a combination of in vitro (laboratory) experimental systems including isolated cells or tissues from animal joints but there is no substitute for

being able to explore the complex processes that occur in the joint in the live animal. It is also the case that we validate all our mouse findings in human disease samples to check that pathways important in the mouse upon disease induction are relevant to the human condition. We are working towards development computer models of OA to guide our research too, though these are in their infancy.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have collaboration with experts using this model already and the information from them allows us to know that relatively small nal measures you will take to minimise welfare costs (harms) to the animals.

Refinement

The surgical model of OA that we use is extremely well tolerated and produces a very robust, though insidious, disease (like the human condition). This model is scored largely by examining histological sections of joints after the mice have been killed but we will explore new methods that are more quantitative and which should help to shorten experiments and possibly reduce numbers.

Animal suffering will be minimized by performing surgery under sterile conditions according to published guidelines from the Laboratory Animal Science Association. Animals will be housed and cared for in groups, with the environment made more stmulating by the addition of so-called 'enrichment' devices. Animals will also receive analgesics after surgery and have deeper bedding during post-surgical recovery.

PROJECT 197

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Regulation of Skeletal Mass and Architecture
Key Words	Bones, loading, exercise, cancer, arthritis, metastasis
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall purpose of this programme of work is to improve the understanding of how we maintain a healthy skeleton, and in particular the response of the skeleton to various stimuli, either alone or in combination. The ultimate aim is to identify and develop new drug targets to maintain a healthy skeleton throughout the life course, and/or combat detrimental physiological changes that occur in diseases.

Specific objectives include:

1) Understanding the effect of altered loading of the skeleton during physiological activity or structural changes (models in which loads are applied to or removed from the skeleton artificially) throughout the life course and during disease.

2) Understanding interactions between abnormal bone and joint loading and other physiological stimuli (i.e. hormones, cytokine or signalling molecules, implants and implant materials) throughout the life course.

3. Understanding interactions between disease states (either natural or via genetic manipulation, and especially cancer) and skeletal homeostasis throughout the life course.

4. The consequences of specific changes in genetic constitution on skeletal homeostasis throughout the life course.

5. The fit between experimentally derived data and computer models of skeletal homeostasis throughout the life course.

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 advance the scientific understanding of the factors controlling normal bone maintenance and bone diseases. Musculoskeletal problems as well as primary and secondary bone cancer have devastating effects on humans, with a significant health economic burden to the UK and beyond. Overall, our research aims to benefit humans by identifying new drug targets to help improve the quality of life and mobility of people with bone and joint problems, and associated bone diseases.

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

The project will use approximately 5500 mice over the five year period.

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

The severity will never exceed moderate for any procedure/protocol. Standard laboratory mice, immunodeficient mice and genetically altered mice will be used where the latter does not show adverse effects due to the alteration. Some animals may undergo surgical procedures, including surgery to induce arthritis and to remove the reproductive organs to mimic the effects of postmenopausal osteoporosis and osteoporosis in men with hormone deficiency. Adverse effects such as problems with wound healing and post-operative pain may be encountered as the result of these procedures. Some mice may also undergo bone loading - adverse effects of this may be pain or lameness. Some animals will be injected with tumour cells and tumours allowed to form. The size of growing tumours will be carefully monitored and not allowed to significantly affect the wellbeing of animals. Some animals will be treated with drugs or other substances to test their effects on bone and or tumour growth. Controls are in place to minimise and/or deal with any adverse effects that may arise as the result of the procedures. All animals will be humanely killed at the end of experiments

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Modelling the whole interaction between bone and or tumour cells and the environments in which they grow in patients is not possible in cell culture. The interventions and procedures needed to understand these interactions cannot be used in patients themselves. Where possible when the effects of agents on single populations of cells (bone or tumour cells) are evaluated, this will be done *in vitro*.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The systems we are using have been used in previous studies and we have done statistical calculations to define the minimum numbers of animals required to obtain significant data. Where possible we will share samples with other projects –this is common practice within our laboratory. We will also use repeated *in vivo* measurements over time where possible to reduce the number of animals needed to obtain statistically meaningful results.

Refinement

Explain the choice of animals 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.

Refinement

We have chosen mice as it is the lowest order of mammal that is still physiologically, anatomically and genetically similar to the human. Mice are the appropriate species for this work, because it allows the use of mutant and transgenic mouse models. The animal models we are using have all been previously validated and published before, and we have refined the protocols over many years of experience. The welfare of the mice will be strictly monitored and we are using protocols that do not produce excessive trauma or suffering. Appropriate pain relief during our protocols will be achieved through appropriate levels of analgesia.

PROJECT 198

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Drug and immunity studies
Key Words	Cancer, leishmaniasis, drug treatment, vaccination,, phototherapy
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aims of this project is to identify new drugs and identify delivery systems that can target a drug to where it is required to cure a disease, using formulations that can be given by a non invasive route. Studies will focus on treatment of cancer and leishmaniasis as these are diseases that cause significant mortality worldwide and they have well established animal models.

In addition, studies will be carried out to identify a novel vaccine that can be used to prevent leishmaniasis and determine what immune responses are associated with protection and the effect of phototherapy on the progression of cutaneous leishmaniasis, either alone or in combination with drug treatment.

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)?

Leishmaniasis is an important neglected disease, the World Health Organisation estimates that there are 900,000-1.3 million new cases/year and 20-30,000 deaths occur each year. Cancer is a major health problem and in 2012 there were 14.1 million new cases and 8.2 million deaths. There is a need for new drugs, better ways of using existing drugs and a vaccine to prevent leishmaniasis. In addition, there is a requirement for non-invasive treatment methods that can combat drug resistant strains, and our studies using phototherapy should address this unmet need.

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

This study will use adult rats, mice and hamsters. Animals are used to (i) provide parasites for studies, (ii) determine drug levels in treated animals (pharmacokinetic studies), (iii) determine the effectiveness of the novel drug formulations (efficacy

studies), (iv) to determine the effectiveness of vaccine formulations, and (v) determine the efficacy of phototerapy against cutaneous leishmaniasis. Over the 5 years of this project the estimated number of animals to be used is shown below. Provision of parasites: mice (200) and hamsters (300) Pharmacokinetic studies: rats (500, mice (800), hamster (250) Drug and phototherapy efficacy studies: mice (800), hamsters (250) Vaccine studies: mice (600), hamsters (250)

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?

In the majority of experiments animals will experience mild adverse effects e.g. discomfort from treatment using a nebuliser or single intravenous injection. Novel drugs are screened against cell lines to determine their potential toxicity and compounds with high toxicity are removed from studies. Pilot studies using a few animals, usually 3, are used in initial in vivo studies to determine unexpected toxic effects. Vaccines tested are based on proteins produced by the parasite/cancer cell so they are part of the normal disease. Leishmaniasis have well documented clinical symptoms e.g. painless lesion formation, increase in liver and spleen weight. We have clear guidelines on when an experiment must be terminated e.g. maximum size of a lesion and animals are terminated by a schedule 1 procedure. Phototherapy would allow non invasive way to treat of skin lesions in cutaneous leishmaniasis and would be effective against drug sensitive and drug resistance strains. It may cause skin heating but people are already exposed to high skin temperatures as part of ebery day life in endemic areas of the world. We have clear information on what is the maximum temperature to cause a burn and we will monitor skin temperature in experiments to ensure we do not reach this temperature.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Unfortunately our current *in vitro* systems cannot mimic all the conditions that occur in the body so whole body experiments are required. Many drugs are less effective because they do not reach all the sites of infection or they are not present for long enough at an infection site. Therefore drug delivery studies that take into account different blood flow different sites within the body or how an infection alters blood flow are required. Similarly the immune system is very complex and multiple cell types and molecules such as cytokines are all involved in immunity to an infection, and the relative importance of each can vary over the course of infection. Therefore immunological studies often require an animal model system to look at how a vaccine/immunotherapy impacts on an infection.

In the case of cutaneous leishmaniasis there are no skin explant model systems as the parasite lives within macrophages within the skin, so there are no suitable models for a mixed cell system where cells travel between skin layers and throughout the body, in response to cytokines/chemokines and expression of surface ligands on multiple cell types.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

It is important that enough animals to show a significant difference between treatments are used. This ensures we get reliable and repeatable results. We use data from past studies carried out in-house or in published studies to ensure that we use enough animals to get valid data.

Refinement

Explain the choice of animals 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.

Refinement

We use bioluminescent cells – for both cancer and parasite studies – in our experiments. This allows us to track the infection in each animal over time. This has allowed us to get a lot more detailed information out of our studies. We have novel drug resistant lines of *Leishmania* parasites which allow us to ensure that the novel drugs we are developing can be effective against parasites that are resistant to drugs currently used to treat people.

PROJECT 199

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Purinergic receptors as treatment targets in muscular dystrophies
Key Words	Duchenne muscular dystrophy, mdx mouse, P2X7, purinoceptor
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Duchenne muscular dystrophy (DMD) is the most common and lethal inherited muscle disorder affecting 1 in 3500-5000 males. Affected boys demonstrate first symptoms in their early childhood; develop progressive muscle weakness and wasting leading to a severe disability (in early teens) and death at the age of 20-30. No cure or treatments are available. This project is directed at further elucidating the link between DMD and abnormalities of specific signalling molecules called P2 purinoceptors, which we have demonstrated to be associated with the dystrophic phenotype. DMD muscles are prone to mechanical damage: This releases intracellular content, including much ATP, which activates P2 receptors. Their activation damages DMD muscle further still. ATP also promotes muscle inflammation, causing additional injury, leading to a vicious cycle of muscle damage and death. Previous findings in the most commonly used animal model of DMD indicated that blocking one specific P2 receptor reduces the disease symptoms. Therefore, we shall study whether pharmacological blockade of P2 receptors can limit DMD progression and thus be a good therapeutic target.

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 successful completion of this project shall lead to a new treatment for otherwise highly debilitating and ultimately lethal disease. This approach already attracted attention of companies interested in re-purposing their P2 antagonist for the treatment of this orphan disease.

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

We propose to use the established mouse models of muscular dystrophy in which we originally identified this abnormality. 1000 mice will be used over the 5 year period. Moreover, we will use a mouse strain that is like a wild type but has a modified purinergic receptor P2RX7, which can be silenced by simply crossing these mice with the established strains producing Cre-recombinase in specific tissues only. 3120 mice will be used 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 levels of severity? What will happen to the animals at the end?

Transient discomfort from the handling and the administration procedure. Bruising at the site of injection. Inadvertent dosing into lungs following oral administration. All these are very rare and animals will be observed daily and any complications would be dealt with in the normal veterinary manner. The overall severity level is mild. Animals will be killed at the end of the experiment.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animals are essential for this project as dystrophic pathology results from a complex interplay between muscle degeneration and regeneration and immune and inflammatory responses, which contribute to both muscle damage and repair. Therefore, we cannot study these complex interactions and the efficacy of the purinergic blockade in an *in vitro* system.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

In all our experimental designs we have been following the ARRIVE guidelines, as demonstrated in Sinadinos et al., 2015. We will keep the numbers of mice used to the minimum consistent with the aims and, wherever feasible, material from individual animals will be used for multiple analyses e.g. serum for creatine kinase assays, diaphragms for organ bath analyses and leg muscles for other analyses. Much of the work will involve histological sections. These will allow for a number of different analyses to be made in each individual animal (e.g. muscle morphometry and immunological infiltrations analyses).

Refinement

Explain the choice of animals 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.

Refinement

There are several mammalian models of DMD: mdx mice strains, two dog, one pig and one cat models. The characteristics of each phenotype and pathology have been analysed for pathological similarities to the human disease and costs of the maintenance. There is a consensus amongst experts that the most appropriate model to test efficacy for DMD are the mdx mouse and the golden retriever muscular dystrophy (GRMD) dog model. Mouse model has been chosen as it allows comparing the results of antagonist administration with those obtained in double knockout studies and to compare P2 treatment efficacy to other therapeutic modalities already described using the mouse model.

The tests to be used in this study are based on specific guidelines provided by TREAT-NMD, designed specifically to standardize experimental protocols that are used as efficacy readouts to allow comparisons of parallel efforts. Following an extensive consultation process TREAT-NMD identified a limited number of experimental protocols, which are appropriate for use in preclinical work and accelerate the development of new therapeutic modalities.

Importantly, in this study pain or distress are not a necessary concomitant to the validity of the experimental outcome. Therefore, animals will be observed and scored according to the checklist established in the initial experiment. Following administration, any animals showing signs of unwanted effects other than those resulting from injection itself would be killed by Schedule 1 method and if multiple animals display such symptoms the experiment with the particular drug will be terminated.

PROJECT 200

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	THERAPEUTICS IN OBESE PREGNANCY AND ITS IMPACT ON OFFSPRING METABOLISM AND HEALTH
Key Words	mouse, pregnancy, obesity, developmental programming, metabolism
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;
No	(iii) improvement of the welfare of animals or of the production

	conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Over 20% of women of childbearing age are obese or overweight. This increases the health service cost during pregnancy. In addition obesity in pregnancy has harmful effects on the woman’s health and the future health of the offspring. The overall aims of the study is to identify potential therapeutic interventions in pregnancy complicated by obesity and gestational diabetes for a successful pregnancy and prevent or reduce offspring susceptibility to obesity, type 2 diabetes and other metabolic diseases in later life.

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

This project will firstly identify physiological changes in both the obese pregnant mother and the developing fetus that may be linked to offspring susceptibility to obesity and type 2 diabetes in later life. The project then aims to develop treatments during pregnancy that will not only result in a successful pregnancy but may also protect the offspring in later life to the harmful effect of eating an unhealthy diet. The outcomes from studies in this project potentially have huge application to develop new preventative and therapeutic treatment in obese pregnancy, which is beneficial to both the mother and her child. It therefore tackles obesity and diabetes across generations, thus reducing the global burden of disease.

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

2500 mice of all ages 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 levels of severity? What will happen to the animals at the end?

We are purposely making the mothers obese during pregnancy as well as the offspring in postnatal life in order to test the effectiveness of the proposed treatment during obese pregnancy. We however are only expecting some mild adverse effect of when making these animals obese, and the level of severity for rest of the procedures are considered moderate, including surgical implantation of an osmotic minipump or an intracranial cannulae. All the animals will be killed at the end of the study and tissues will be samples for analysis.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

It is unethical to induce obesity during pregnancy in humans and to analyse its effect on the fetus. It is also unethical to treat obese pregnant women without first finding out how these treatments may be affecting the developing fetus. Furthermore, it will take years in humans to find out how treatment in obese pregnancy affects the health and disease susceptibility of the offspring in later life and into aging so using mice with their short lifespan will enable us to find out the long term effect of the treatments during early life. Our mouse studies potentially have direct translational application to developing new preventative treatments for the harmful effects of obesity and gestational diabetes in pregnancy, which may have beneficial effects on the offspring in later life.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We use statistical power calculations to determine the ideal number of animals to use in our experiments. The experimental design that we will use will allow publication of the results according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. This will maximise information published and minimising unnecessary duplication of studies.

Refinement

Explain the choice of animals 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.

Refinement

We chose the mouse as our animal model because modifying their nutrition during pregnancy, simulating those experienced by humans, has already been show to result in obesity, diabetes and fatty liver condition resembling those observe in humans. The advantage of using mice it that it has a short lifespan so we can study what happens to the newborns through to adulthood and into aging in a short duration of time. In addition, a large variety of genetically modified mice are available to study human disease that is still lacking in other animal models.

PROJECT 201

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Developmental Programming in Birds: Mechanisms and Interventions
Key Words	Development, Pregnancy, Hypertension, Disease
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;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Heart disease is the greatest killer in the world imposing a substantial burden on every nation's health and wealth. Therefore, it is an expensive as well as important problem to resolve. Although we have known for decades that our genes interact with traditional lifestyle risk factors, such as smoking and obesity to set an increased risk of heart disease, it has now become clear that the gene-environment interaction before birth may be just as if not more important than the gene-environment interaction after birth in setting a risk of heart disease. [REDACTED] The aim of this project licence is to understand the mechanisms involved, how different adverse conditions during developemt may interact, and to identify potential translation to human clinical intervention.

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

The work in this licence is to be done in the chicken. The reason why chickens will be used is because in contrast to all mammals (except monotremes), the chicken is the only established animal model in which the direct effects on the offspring of adverse early environmental conditions, such as alterations in oxygenation, nutrition or exposure to stress can be isolated, completely independent of effects on the mother and/or the placenta. Establishing direct effects on the developing offspring of adverse environmental conditions means we can better isolate mechanisms involved and better focus on interventional strategies to guide future translation into human clinical practice. The proposed work may thus hasten translation to relatively simple

but novel human clinical interventions to not only treat the mother, but also her progeny. This will reduce the burden of developmental origins of heart disease, thereby having a major positive clinical, economic and societal impact on health.

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

We will use chickens at different stages of the life course. We expect to use 3000 adult/juvenile birds, 2500 hatchlings and 10000 chick embryos 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 levels of severity? What will happen to the animals at the end?

Imposing adverse conditions during incubation (e.g. reducing levels of oxygenation proportions to lower than normal) will impact of the development of the chick embryo in such a way that it will increase morbidity and decrease hatchability. This is the essence of the project application. We will test interventions that we hope would significantly protect against this, decreasing cardiovascular morbidity, protecting hatchability and health in later life. In some instances birds will undergo surgery under general anaesthesia to implant instruments in their legs to better monitor their cardiovascular system. While most will make an unremarkable recovery after surgery, a small proportion of these birds may have difficulty standing on both legs by 24h after surgery. These birds will be closely monitored with advice from the named veterinary expert. The severity for the project licence will be moderate, which is the least severity band when some animals will undergo surgery. Animals at the end of protocols will be killed and tissues used to obtain data.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In some experiments, we must use whole animals because how our organs and systems function, for instance the cardiovascular system, is regulated by complex networks of circulatory, organ, cellular and molecular interactions, none of which have been reconstituted in models. The overall system is not well enough understood to make mathematical modelling useful, except in extremely limited circumstances.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To ensure the minimum number of animals is used in this project to address all objectives, we have considered the choice of species very carefully. The real ethical, biological and economic value of birds relative to other experimental animal models, such as rats and mice, can be best appreciated by considering the lack of need for surrogate mothers or for control of the effects of litter variation or the effects on lactation, as is needed in mammals that give birth to litters and suckle their young. For example, in rodents, one litter irrespective of the number of pups in that litter, is considered an n=1 for many statistical comparisons. In addition, adverse pregnancy in mammals may affect the quality of the mother's milk for lactation. Therefore, in rodents, additional control animals, such as surrogate mothers for newborn pups, are needed to understand these effects better. By using the bird as the species of choice to address the project objectives, this markedly reduces the number of animal groups, as there is not need to control for litters or maternal milk. Therefore, this significantly contributes to the 3Rs principle of reduction as enshrined in EU Directive 2010/63.

In science, power calculations determine the minimum number of animals needed for any experimental outcome. These power calculations in this application reveal that we need a mimimum number of n=8 per outcome measured per group.

To minimise failure and improve success rate in birds which require surgical preparation, we will keep the surgery procedures to a minimum and draw experience from expert collaborators.

Refinement

Explain the choice of animals 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.

Refinement

We have refined the project such that a large component of the work can be achieved by investigating isolated organs and tissues rather than in the live animal. Smaller components of the work will involve studying whole animals under terminal anaesthesia, or conscious animals which have been surgically prepared under general anaesthesia. It is necessary to study conscious animals as anaesthesia has potent effects on physiology, for instance depressing the cardiovascular system. On these occasions, experiments in conscious animals will only be performed following appropriate post-surgical recovery. We will keep suffering to the minimum by using procedures with the least possible severity, and by subsequent monitoring with veterinary advice.

With experience from the previous project licence, we have significantly streamlined and refined surgical procedures to improve surgical techniques that reduce bleeding, shorten anaesthetic exposure and improve port-surgical recovery. In some preparations, we will need to catheterise a tributary of the leg artery of the adult bird by isolating the vessel on the outside of the leg muscles rather than through an inguinal approach. From past experience, we have found that this refined surgical approach markedly reduces the disruption of associated circulations and improves the post-surgical recovery of the bird.

In some birds, under recoverable anaesthesia and strict sterile conditions, catheters will be placed in one leg and a flow probe in the other. Following 5 days of postoperative recovery, experiments lasting 7-10 days will be performed. [REDACTED]A score sheet has been developed to assess mobility and behaviour to guide decisions, and it will continue to be used and improved as appropriate. Pain relief will be provided as appropriate.

PROJECT 202

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Safety and efficacy of hepatic regenerative medicines
Key Words	Liver, Injury, Regeneration, Repair, Stem cell
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Liver disease in the UK is increasing in prevalence. Novel medicines have the ability to promote tissue repair and aid liver regeneration (to re-build and gain function). However, need rigorous testing before being applied to humans. The aim of this work is to assess the safety and mechanism of action of novel therapies to promote hepatic regrowth by finding out 1. How liver disease occurs, 2. How the liver repairs itself and 3. What happens when regrowth goes wrong? By understanding these events we can better design and use safely drugs that can prevent 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)?

The benefits of this work primarily focus on liver disease and the treatment of such. However many of the processes which occur during liver disease also occur during disease in other tissues so our work in the liver will contribute to disease progression in a range of organs. The impact of techniques for the development of tracking regenerative medicines (cells, biologics) by imaging has a far wider benefit as these therapies have the potential to be utilized in curing a vast number of diseases. The translation of the novel imaging methods to assess liver disease can also be applied to other diseased tissues so our work will contribute to disease progression in a range of organs

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 the five years. We will use a maximum of 7900 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?

As we are modelling disease our animals they will progressively demonstrate symptoms of the disease. However, using imaging and non-invasive blood based biomarkers we can closely manage these signs and generally can define disease at a much earlier stage and therefore we ensure that the animals do not undergo any undue suffering

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Regeneration (or regrowth/re-establish function) in the liver is a complex, multi-staged process in which there are any different cell types interacting with one and other. It is impossible to model such complexity without using animal models and an understand of potential safety implications for human health must be established in animal models first.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

It is possible to calculate the numbers of animals required for experimentation based on data from previous data. Imaging and non-invasive blood-based biomarkers lets animals be used as their own control, allowing paired comparisons. Moreover, imaging is inherently sequential, increasing statistical power and using fewer animals to achieve the same statistical power as conventional designs. In all cases we ensure that we have calculated the minimum number of animals required for the experiment to give us useful data. This approach reduces the animal numbers required, and also reduces the likelihood that the animal experiment would have to be repeated.

Refinement

Explain the choice of animals 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.

Refinement

Using imaging non-invasive blood-based biomarkers we regularly refine the disease models we use to reduce animal harm and to improve the effectiveness of our models. We can also stage disease and stop experiments before external clinical

signs appear, thus limiting disease severity. Because of this we can ultimately use fewer animals per procedure to and still generate meaningful and clinically relevant data. We also regularly monitor body weight, body condition, food and fluid intake of animals as a measure of disease; we set strict limits to ensure that there is limited harm to the animals used.

PROJECT 203

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Combination Therapy for cancer
Key Words	cancer research, combination therapy, oral drug delivery
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall objective of this project is to develop better cancer treatments which combine different drugs and radiotherapy in new ways.

These treatments when combined in the right way and in the right order will make the cancer cells die more effectively but will cause less harm to normal non-cancerous parts of the body and should cause less side effects

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

Despite the incidence rates for cancer in the UK stabilising in the last decade, 1 in 2 people will develop cancer in their lifetime. Notwithstanding recent breakthroughs in treatments for a few specific cancers (testicular cancer, lymphoma, breast cancer) mortality rates remain high and many drugs have lots of side effects because they also kill or damage normal healthy cells. New treatments which work better on killing the cancer and which have fewer side effects are urgently required. Currently, radiotherapy is utilised in the treatment of approximately 40% of cancer patients, however this procedure could benefit many more patients and could elicit cures if it were administered in a more targeted fashion which resulted in giving more radiation to the tumour and less radiation to normal cells. This would improve tumour kill levels without the problem off side effects to normal cells which is the main reason for the failure to cure cancer with radiotherapy. This aim of the proposed studies is to invent new treatments using not single drugs or treatments on their own, but combining drugs and different types of radiation to make them home in on and kill cancer cells but not hurt the normal healthy tissues. This approach has already led to new less toxic treatments for cancer in the clinic and further research will improve cancer

treatment by killing the cancer more effectively and reduce toxic side effects. The animal studies we have previously undertaken have contributed to the clinical translation of treatment schemes into patients. Continuation of this work will guide the design of better treatments which will be utilised in cancer patients in the longer term. Thus any good treatments we discover from these studies will inform clinical practice and will be to the benefit of cancer patients and health care providers. The project may also lead to the generation of more affordable treatments as many of the drugs we are using as part of the treatment are more common drugs which cost much less than many of the new cancer treatments. This will benefit health care providers and patients as more affordable treatments can be more widely utilised at a lower cost. The way we are using radiation and drugs is also new and could provide basic scientific knowledge that can be used by other researchers and clinicians to develop other treatments and it will add to the knowledge we have about what happens to cancer and normal cells when they get irradiated. This knowledge is very important for cancer research scientists as we can make more and better treatments if we understand how drugs and radiation affects cells we can understand why some treatments don’t cure cancer and improve upon them.

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

For investigations using cancer cells, vertebrates must be used. For these studies rodents will be used as mice and rats are the lowest vertebrate group. Many cancer models in mice are well characterised and accepted for cancer experimentation. Rats will be used only to undertake drug delivery experiments using oral thin Films (OTFs) as larger rodents are required both to administer the film on the tongue and allow withdrawal of blood. The use of live animals is required to (a) examine the impact on tumour growth of combinations of therapies; (b) model the distribution of drugs and radioactive molecules so we can tell if we can get enough in the tumour to have an effect and (c) assess the behavior of the drugs in a live animal which may be very different to what happens in a laboratory as bodies are much more complicated than cells in a dish in a laboratory. No more than 3500 mice and 500 rats will be used. However this is the maximum number of animals and it is probable that much fewer numbers will be used.

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?

In all the planned experiments we have chosen the least severe way to do the experiments and get the data we need to achieve our aims. In all cases we will do lots of experiments in the laboratory before we do any animal experiments and use the least severe models we can to still achieve the aims and ensure no animal lives are wasted. The protocols are all of moderate severity limit but this is really the worst case scenario and actually most of the experiments have only mild severity. In some experiments we will use anesthetic to make the animals sleep during the

experiments this is to reduce any short term pain they may experience but also to make sure they are not stressed by procedures. In less than 1% of cases animals die because of the anesthetic but all precautions will be taken to make sure this does not happen and animals will be well cared for and monitored throughout this process. In a few cases we will need to perform surgery on the animal- these will be performed while the animals are under anesthetic to minimise stress and pain. These techniques will be performed only by very experienced people and if any infections or swelling or redness occur at the sights of surgery the veterinarian will be called to treat the animal or the animal will be euthanised if they cannot be treated. In some cases cells or drugs or agents will be injected into the animal which may cause a transient discomfort but again using only well trained and experienced people will minimise this adverse effect and the volumes and routes of administration are chosen to cause the least possible discomfort. The growth of the tumour on the flank of the mice after injection of cells does not cause the animal any adverse effects however mice who have metastatic cancer may be unwell , however the experiments will be stopped before this causes major harm and the wellbeing of the animal will be monitored carefully and very regularly. Any animals that show signs of illness, weight loss or lack of general health and wellbeing will be euthanised. Furthermore any metastatic models or models where the cancer is injected into the animal rather than on the flank will be trailed in a small number of animals so we know exactly what the course of the disease will be and can ensure animals are euthanised well before they experience unacceptable adverse events. The drugs and agents we will be giving to the animals are mostly well characterised and have been used in other experiments and doses that are safe are reported in the literature. These doses will be used for our experiments. However it is possible that some agents may cause adverse effects. Therefore any new drugs or agents alone or in combinations will be tested first in a small number of mice using a protocol called dose range finding studies. This lets us try a few doses based on the levels of similar drugs in the literature to ensure they cause minimal harm before giving this drug or combinations to a larger group in the therapy studies. If any drugs or combinations cause unacceptable weight loss ill health or adverse effects they shall not be used in animal studies. In some cases we may need to give agents by mouth and to do this we use oral gavage where agents are fed directly into the stomach through the throat. This approach while less severe than injection hold the possibility of causing irritation in the throat but again only experienced people will do this experiment and animals will again be monitored continuously following this procedure and any suffering terminated by euthanasia. Occasionally animals will be restrained during procedure. Only well accepted methods of restraint will be used using ways that minimise stress. Most animals quickly become used to the restraint but if there is signs of distress animals will be euthanised before the procedure. The effects of radiation and radiolabelled drugs on animals are very well documented and through our own previous 20 years of work and other published study we know that the radiation causes minimal adverse effects to none in the animals, If any animals show

signs of ill health or lack of well being they will be withdrawn from the study and euthanised. Blood samples may be taken from the animals this may on rare occasions cause anemia or other blood problems. To avoid this no more than 10% of the animals blood volume will be withdrawn on any one occasion and the guidelines for blood sampling will be followed. Again animals will be monitored at all stages of the process and any animals showing adverse effects will be withdrawn from the study and euthanised. Most of the mice used in the study have been bred to not have an immune system as this is the only way we can grow human cancer cells in the mice so their bodies will not reject the cancer. The animals are kept in sterile housing with sterile food and water to avoid the mice being exposed to possible infection and any procedures will be done in a sterile environment to avoid infection. Occasionally we may need to withdraw food and drink from the animals but this will be transient and again will adhere to all guidelines. The animals may be kept in special cages that allows us to collect feces or urine but adverse effect are unlikely and this protocol is considered moderate. The animals in these cages will be continuously monitored and any animals showing signs of discomfort or stress will be returned to their home cages. For rats who are being given oral thin films on their tongue there is a small possibility of irritation or discomfort in the mouth. This is limited by the animals being anesthetised before we insert the film and the animals being monitored closely after insertion for any signs of choking or pain. Any animal which shows sign of choking or breathing difficulties will be euthanised immediately.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

For investigations using cancer cells, vertebrates must be used. For these studies rodents will be used as mice and rats are the lowest vertebrate group which are well characterised for the establishment of models that are accepted for cancer experimentation. Rats will be used only to undertake drug delivery experiments using oral thin Films (OTFs) as larger rodents are required both to administer the film on the tongue and allow withdrawal of blood. The use of live animals is required to (a) examine the impact on tumour growth of combinations of therapies; (b) model the distribution of drugs nad radioactive molecules so we can tell if we can get enough in the tumour to have an effect and (c) assess the behaviour of the drugs in a live animal which may be very different to what happens in a laboratory as bodies are much more complicated than cells in a dish in a lab.

[REDACTED]This has enables us to replace animal experimentation for every idea we have to doing the experiments in the laboratory first and using these models instead of animals to find the best treatments.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To reduce the number of animals utilised, all studies will be scientifically robust and apply appropriate statistical methods (under advisement of University of Strathclyde Statistics department). All studies are first carried out in cell models not using animals and only promising treatments are carried forward for experimentation in the least severe model which is growing the cancer on the flank of the mouse (xenografts). Only treatments which show promise in xenografts will then be evaluated in the slightly more severe models of metastases where the cancer is spread throughout the body of the mouse and models where the cancer is grown in the organ which it would normally grow (orthotopic) The experimental design is organised to allow us to look at multiple treatments at the same time within one experiment. This limits the number of animals utilised in control groups and the data generated allow direct statistical comparison that is meaningful, based upon internal controls. The numbers of animals proposed are the minimum which will allow rigorous statistical analysis and are large enough to preclude multiple repetitions. These numbers are based upon both our previous investigations [REDACTED] and the advice of statisticians.

Some studies have been specifically designed to use smaller number of animals for example to let us look at how tumours are metastasising we will use a technique called bioluminescent imaging. In this experiment the cells we inject into the mouse have a gene added which allows us to use a type of camera to track the cells and their growth (bioluminescent imaging). The use of this experimental set up allows us to look at spread of cancer in one mouse over time without having to sacrifice the mouse to look at the spread following dissection. Range finding studies are designed to look at the effect on the animals of drugs and agents that we have limited published data or experience with so that any potential adverse effects would only occur in a small number of animals rather than going straight to larger groups in the therapy studies. For studies looking at how the drug gets around the body or is released (PK studies) from new formulations this experiment will be designed using repeated sampling so the same rat will be utilised for blood draw and analysis at multiple time points. This approach has been utilised in previous experiments on an alternative animal licence successfully with only moderate effects on the animals and this enables extensive PK data to be collected from a smaller number of animals.

Overall the data will be assessed using the correct statistics that are best for the individual experiments and animal numbers will be set using calculations that tell us the minimum number of animals that are needed to let us get the data we need. This will be done with the statistician to ensure that all experiments use the minimum number of animals to give us robust and accurate data.

Refinement

Explain the choice of animals 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.

Refinement

We will use mice without an immune system (athymic nude and SCID mice) for the majority of studies (with the exception of PK studies), the only models suitable for establishment of human cancer models. We will also, where possible, utilise wild type mice, limiting the need for genetically modified species. [REDACTED]

Detailed molecular and genetic characterisation is now available for human cancer cells and these sources will be employed to ensure suitable models are utilised for the correct scientific purpose they are used for. Furthermore all cancer cell lines utilised will be checked to make sure they are free of infections which would affect the animals and that cells lines are what they are supposed to be so no animals are wasted in experiments which are wrong. .

Suffering will be minimised by:

- (a) Using all available published knowledge and experience to predict adverse effects;
- (b) Provision of appropriate environments for animal maintenance;
- (c) Adherence to local guidelines and the “Guidelines for the use of animals in cancer research (2010)”;
- (d) Only experience licences will undertake procedures and continued training and monitoring will be undertaken by the licence holder and NACWO to ensure best practice.

If any animal demonstrates signs of distress or ill health the Named vet and NACWO will be consulted and appropriate action taken.
For dose-range-finding experiments, mice are administered with increasing amounts of agents. While this leads to more interventions and perhaps enhanced adverse effects in a small number of animals, we believe that these are minimal and the experimental design will allow significant reduction in overall mouse numbers required.

Animals being removed from cages to undergo experimental procedure will be anaesthetised and handled at all times according to standards for best practice and local guidelines thus ensuring their insentience during procedures and minimising distress during anaesthetic recovery.

All protocols have moderate severity limits. Systemic and orthotopic models will only be used for schemes with a high chance of success. Humane endpoints will be used instead of survival which has a higher severity level, and these will be refined in light

of experience and according to local and published guidelines. Protocols will be put in place to monitor the condition of the animals using a scoring system to assess their health and well being. Serial termination of a small number of animals will be employed when optimising new systemic tumour models, in order to detect and profile internal tumours and confirm BLI imaging for subsequent studies. This will also allow modification of subsequent experiments to minimise animal suffering.

PROJECT 204

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Associative analysis of recognition memory in an animal model of Alzheimer's disease.
Key Words	Association, Recognition, Memory, Alzheimer's, Mouse
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Early diagnosis of Alzheimer's disease (AD) is a research priority. A psychological test that could identify AD would be ideal, as such tests are cheap, noninvasive and easy to administer. But currently such tests are crude - and identifying new *cognitive markers* of AD in humans is lengthy and expensive, requiring repeated screening of many participants, only a few of whom will develop AD. Thus mice that are genetically modified to partially mimic AD pathology are often used instead. Although they are only an imperfect parallel to human AD, they develop symptoms rapidly, so their cognitive changes can be studied over a short period. Thus we have been studying recognition memory in one particular, extensively researched genetically modified mouse model of AD, in which the early stages of the disease develop between 4-5 months.

We use a recognition task relying on rodent's natural curiosity. If mice explore an object and are then permitted to explore it *and* a second, novel object, they preferentially explore the novel object - suggesting they *recognise* the other. We interpret this behaviour in terms of *associative theory*, which explains recognition in terms of two processes, *self-priming* and *retrieval-priming*. *Self-priming*, stems from having previously experienced the stimulus, and *retrieval-priming* is produced by expectation (prediction via associative learning) of the stimulus.

Our strain of mouse is unimpaired on this task at 4-5 months - but our theory can explain this. For example, if recognition relies on two processes, it is possible that only one is impaired. Thus recognition might *seem* normal, but more subtle tests might reveal a deficit. We found evidence supporting this: measuring *self-priming* and *retrieval-priming* separately we found a selective deficit in *retrieval-priming* in 4-5-month-old mice. This result is promising - but there are alternative explanations of

these findings. We **aim** to rule out these alternatives, and establish definitively whether or not there is evidence for an impairment in either *self-priming* or *retrieval-priming* in the 4-5-month-old transgenic animals.

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 short term this work will increase understanding of how cognitive impairments develop in AD. Also - as our associative analysis of recognition is novel - it will be of interest to those working on the theoretical basis of recognition. In the medium term this work will provide information about the potential recognition deficits in patients in the early preclinical stages of AD, which could in the long term result in a new, diagnostic test for AD.

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

We anticipate using no more than 320 genetically altered mice 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 levels of severity? What will happen to the animals at the end?

The experimental procedure involves placing animals into an arena and allowing them to explore small, domestic junk objects (e.g. salt cellars, ashtrays etc) while they are video-recorded. The only adverse effect we have seen is mild stress on initial placements - evident as a tendency to freeze and defecate. Both are typically confined to the first time the mouse is in the arena. Thereafter the mice seem relaxed -- critical, as the success of these experiments relies on the mice being willing to freely explore the objects presented. The main potential adverse effect of this project stems from the use of this strain of transgenic mouse. In common with other strains with these mutations it has an elevated mortality rate (10.5% in our lab). These deaths are sudden, and we have never yet experienced any sign that the animal suffers beforehand; moreover these deaths are not associated with post-mortem pathology. We have no evidence that mortality is elevated by the behavioural task - in fact we have never had a mouse die during the course of one of these experiments. At the end of the experiments the mice will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Achieving the objectives requires observation of behaviour, and so we must use *live animals*. Performing the studies in patients at an equivalent stage of the disease is

impossible, as early diagnosis is impossible (developing an early diagnostic test being the motivator for the work).

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will minimise the number of animals by using within-animal comparisons for our key recognition measures, and analysing behaviour over short time periods. We will also use statistical techniques to estimate the minimum number of mice consistent with obtaining a scientifically meaningful result. We will reduce variability by counterbalancing the various objects and replicating key results in the same mice with new objects.

Refinement

Explain the choice of animals 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.

Refinement

Realising the scientific benefit of this project requires us to continue using this specific strain of genetically altered mouse, because all the previous work on which this project is based has been performed in this same strain. These animals do not typically show any adverse clinical signs except for the mild cognitive deficits that we are seeking to analyse.

To reduce the mild stress on initial placement in the arena, we have introduced the refinement of adding a series of placements in the empty arena *before* the start of the experiment proper. This is sufficient to habituate any freezing behaviour during subsequent placements, and our previous work shows that the mice appear relaxed during the remaining stages of the protocol - which may in fact be regarded as a form of environmental enrichment. In the unlikely event that a mouse showed a strong fear reaction to one of the visual stimuli it would immediately be removed from the arena (although we have never encountered such a reaction).

PROJECT 205

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Studies of Cilliopathies
Key Words	Cilia, left-right patterning, PKD, PCD
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Cilia, small hair-like extensions from cells, perform multiple jobs in the body. Some beat, transporting fluid or driving a sperm cell forward, others are immotile and these help cells respond to signals. When cilia fail to function correctly they cause a group of diseases called the ciliopathies that can affect many processes in the body. We are working to understand the role of cilia in three processes: (1) establishing a distinct left and right hand side of the body; (2) preventing polycystic kidney disease; and (3) clearing mucus from the respiratory tract.

Correctly establishing a left and right hand side of the body is important in patterning the organs, particularly the developing heart - as failure can lead to congenital heart disease. This requires the interaction of both motile and immotile cilia in the early embryo. We are working to understand how this interaction takes place and the ways in which it can go wrong – this can lead to abnormal left-right patterning of the body and associated heart disease.

Polycystic kidney disease (PKD) is a genetic disorder that results in end stage renal failure of 50% of carriers by the age of 60. About 70,000 people in the UK are affected by PKD, a life threatening inherited disease that can cause kidney failure and affect other organs in the body (http://www.pkdcharity.org.uk). We are investigating the relationship between the known PKD genes and the cilium. We are aiming to understand how the PKD proteins act when they are in a cilium versus when they are not in a cilium.

Primary ciliary dyskinesia (PCD) results from defective cilia motility, giving rise to both lung disease and left-right patterning defects. This rare inherited disease affects approximately 1 in 15,000. This life-long disorder affects quality of life and in many cases can lead to major lung disease and require lung transplantation. In

collaboration with clinical groups we are testing the role of a number of genes in PCD and creating and studying mouse models of PCD.

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

This research is aimed at extending our understanding of the normal function of cilia in mammalian development and how this goes wrong to cause disease. Major questions remain unanswered about how cilia interact with cilia-driven flow in the early embryo and with urine flow in the kidney. Our research seeks to understand these interactions. The outcomes of such understanding feed into the sum of knowledge of how disease occurs. It provides the basic information that is required to both understand and to ultimately treat human disease. However, any such treatment is beyond this current research. The work will also identify genes that could cause human disease – genes identified in this work become candidates when patients with overlapping signs and symptoms are screened. Not all such candidate genes will carry mutations in humans, but having a list of such 'likely genes' makes it far easier and faster to identify which gene are mutated in people. Finally, mice that model aspects of human disease will be made and characterised in this work. Once characterised they become available for further study of those diseases and may be used to test whether a future therapy is either safe and/or effective.

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

We only use mice – up to 18,500 in 5 years of research. The vast majority of our experiments analyse mouse embryos – adult mice are used to breed genetic alterations and for generating embryos. The numbers we need are mainly dictated by the small size of the mouse embryo – which does not yield much tissue for study – and by the need assess complex phenotypes where a single mutation can gives rise to a variety of outcomes. We employ statistical tests in both the design and analysis stages of projects.

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

The majority of the adult mice suffer no adverse effects as they simply carry a genetic alteration. We use these to generate embryos that have abnormalities associated with cilia defects: these primarily affect left-right patterning and the kidneys. Small numbers of adult mice will have kidney cysts, abnormal left-right patterning, and/or immotile tracheal cilia. To date we have seen no adverse effects in these mice. It remains possible that congenital heart disease or polycystic kidney disease could occur in these mice, therefore such mice are very closely monitored and advice sought from the welfare officer (NACWO) regarding welfare to ensure the mice suffer only mild long-term or moderate short-term adverse effects. Furthermore,

many of the genetic changes are point mutations designed to only partially inactivate the gene and as such are less likely to develop the full clinical disease.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We are studying the role of cilia in embryonic development and adult physiology. These are complex processes that involve the interaction of multiple tissues, and it not currently possible to model this in cell culture. We routinely complement animal studies with those in tissue culture. However, the need remains to return to mice to verify such results.

The mouse is the simplest mammalian system that we can study. It has excellent genetics, as well as having similar anatomy and physiology to humans. No unprotected organism has a four chambered heart, mucociliary clearance and a mesonephric kidney (as do mammals).

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Regular (monthly) planning meetings help ensure that only the required numbers of animals are bred. These numbers are calculated on the basis of statistical tests that are performed with advice of a professional statistician. Lines that are no longer needed are cryopreserved. We only rarely generate our own GA lines; the use of emerging genome editing techniques (CRISPR/Cas9) should ensure that fewer animals are used than are required by traditional stem cell-based methodologies.

Refinement

Explain the choice of animals 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.

Refinement

Non-mammalian organisms have significant differences in anatomy and physiology from humans, making them unsuitable to model many aspects of the ciliopathies. In particular, mice and humans have a four chambered heart, mucociliary clearance and a mesonephric kidney (as do mammals).

For the majority of our experiments we will analyse embryonic tissue, removing the need to breed affected adults – unaffected adult carriers will be bred. For the adult mice we analyse we will monitor their welfare, in order to prevent or minimise any possible distress. Where appropriate we will use conditional deletion of genes to restrict defects to the tissues we wish to analyse.

PROJECT 206

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Murine models of infection, immunity and therapy
Key Words	Infection, vaccination, antibiotic, treatment, immunity
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aims of the project are to use well characterised mouse models to investigate the influence of mammalian genetics (inheritable characters) on infection and immunity (health and ability to resist infections), including the response to vaccination and treatments, including antibiotics. Here, we are building upon our work under previous licences where we identified bacterial genes that can serve as drug targets and mammalian genes that influence infection susceptibility, immunity and response to treatments. The objectives are:-

1. To build upon our experience of uncomplicated mouse models to further characterise pathogen and mammalian genes that contribute to infection susceptibility.
2. To use these models to further characterise the genetics of how vaccines induce protection against infection, including searching for simple tests to show a vaccine has worked.
3. To investigate the effectiveness of antibiotic treatment in these models where pathogens (bacteria) of different resistance to antibiotics are used.
4. To investigate the impact of antibiotic treatment on host (patient) recovery and on the microbiota (the bacteria that live in and on us and help keep us healthy).
5. To translate these aims into clinical practice.

These aims and objectives are built on well-founded mouse models and a vast amount of experience on how to (a) minimise animal usage and limit any potential suffering; (b) maximally exploit materials taken from such experiments in terms of data generation; (c) match such experiments with related cellular and clinical investigations.

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

There is a compelling need for new approaches to the prevention and the treatment of infectious diseases and the implications of colonising microbes. The threats of antibiotic resistance and new diseases such as Ebola is increasing. Sums of up to a trillion pounds have been quoted as the potential global cost of antibiotic resistance and there is a need to develop better vaccines against these and other infectious agents. We must find alternative approaches if we are to avoid the potential evolution of non-treatable infections and less effective vaccines. The work outlined in this application could bring benefits across a spectrum of areas ranging from (a) identifying new ways to treat antibiotic resistant microorganisms (b) Elucidating the benefits of the microbiota (normal microbe communities in the body) in diseases and treatments (c) the identification of individuals more likely to fail treatment. We believe benefits could be manifested in more cost effective treatment for patients (health and economic benefit), the reduction in circulating ARMs and better use of antibiotics and vaccines (economic), pharmaceutical products (already two spin out companies formed) and improved clinical practice (physicians). Our work under our licences has facilitated the formation of two different companies with the licence holder as a founder. These companies are using approaches invented under our licences to develop products targeting the clinical market. These are based on antibodies and bacteriotherapies.

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

Animals may be used for multiple but related procedures to study the impact of vaccination and treatment and subsequent infection with pathogens. We will use up to 2500 mice over the 5 year period, these will be normal inbred mice or those harbouring genetic modifications.

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

The whole licence is built around exposing mice to pathogens and then treating them, and we employ limited physical interventions to ensure a comfortable environment for the mice. Occasionally, mice may be imaged using light collecting equipment where they undergo no suffering. No surgery is involved in our work and tissue sampling is limited in live mice to blood and faeces. Animals are treated with antibiotics, antibodies, vaccines or immunotherapies that are generally non-toxic to the mice, minimising the risk of any suffering in this context. For pathogen challenge we only use microbial species for which we (or others through extensive peer reviewed publications)) have significant experience. Here, with the doses and routes employed we have an excellent chance of predicting the course of infection and have devised well founded schemes to intervene before mice undergo significant suffering (using careful clinical observation and scoring). If we do encounter any unanticipated problems e.g. rapid unexpected onset of illness, such experiments are not repeated in the same format or in a format likely to give a repeat of the problems.

Over the past two decades, such events have been extremely rare indeed, occurring in ~1:1000 novel mouse lines tested.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

It is not possible to monitor all aspects of the interactions between the different stages of a real infection process or to observe the immune system ouside of whole animals. We recognise that our mouse systems have limitations and we are working hard to link these areas in a meaningful way and are making progress. [REDACTED] We have also dramatically improved our sampling techniques to derive the maximum amount of information from the smallest sample, again reducing the number of mice needed per experiment and improving our statistical power.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The experimental approaches described above have been vigorously evaluated over the past two decades. Analysis is routinely performed in single and controlled experiments that provide a complete picture, thereby reducing the number of animals required. By keeping our experimental conditions and protocols well controlled we are able to perform highly reproducible and statistically meaningful experiments using the minimal number of animals. [REDACTED]During this period we have also established statistical measures linked to biologically relevant outcomes to ensure we do employ the minimal number of animals per experiment.

Refinement

Explain the choice of animals 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.

Refinement

Over the years we have gained tremendous experience with our infection models and through careful observation we are able to minimise the potential suffering of these animals. We have been able to identify key clinical signatures that indicate illness in infected animals and consequently such animals can be quickly and humanely killed. Mice are monitored throughout experiments and we collect daily

scores composed of a set of physical signs. Individual signatures are collected for each mouse and an accumulated score indicates the degree of illness.

We group house our mice as they are naturally social animals, and provide cardboard tunnels and nesting materials to facilitate normal behaviours.

We use minimally invasive sampling, such as sampling from faeces, where possible.

The animal facility has a database for tracking individual mice and the procedures they undergo. Health and welfare are monitored, and any concerns entered onto the database to provide live reporting on the condition of each animal, enabling swift decision-making where there are concerns.

PROJECT 207

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Molecular regulation of the African trypanosome life cycle
Key Words	Parasite, sleeping sickness, trypanosome, differentiation
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Trypanosomes are parasites that cause fatal human and animal disease in sub Saharan Africa. They are single celled organisms, spread by tsetse flies. In the blood of mammals, the parasites monitor their population size by releasing a signal. When there is a critical level of parasites in the blood, the trypanosomes respond by stopping their cell division and preparing for their transmission by tsetse flies. This involves development to a new life cycle stage called the ‘stumpy’ form that can establish the infection in the tsetse gut when taken in during a bloodmeal. [REDACTED]

Trypanosomes also often exist in mammal hosts in mixed infections with other related trypanosome species. We hypothesise that in these coinfections the parasites might detect, and respond to, the signals released by other parasites and that this might shape their infection profile, or select for increased virulence or transmission potential. A further part of our work will explore whether these different trypanosome species can ‘talk to one another’, the mechanisms through which they achieve this and the consequences for their virulence or life cycle development.

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

The discovery of the mechanisms by which trypanosome parasites respond to their environment provides novel routes to controlling the parasite. For example, manipulating the signals that normally drive their cell division arrest could reduce the virulence of the parasite, as could perturbing the activity of molecules that transmit this signal within the cell and between cells. The gene expression profile of some of the molecules linked to arrest as stumpy forms, or proliferation, might also provide

molecular markers that permit the prediction of the virulence of the parasite or their likelihood for transmission. The interactions of different trypanosome species could also be informative in predicting the threat of virulence of parasites that have been selected in coinfected hosts, since they may exhibit particular virulence profiles in the absence of coinfecting competitors. Trypanosomes are amongst the most evolutionarily ancient nucleated cells (eukaryotes). Hence, as well as discovering potential vulnerabilities in their life cycle that can be exploited for therapy, the understanding of their molecular pathways involved in development may provide fundamental knowledge helpful to understand how all cells undergo specialisation, including our own cells.

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

We will predominantly use mice for our experiments. The parasite life cycle cannot be adequately reproduced in cell culture such that much of our analysis of the consequences of molecular disruptions needs to be analysed in a mammal host. However, we have a great deal of experience of growing trypanosomes in mice where the consequences of infection are predictable, allowing us to minimise suffering. Sometimes, we also need to generate very large numbers of parasites (for example for molecular analysis) and this cannot be achieved with cells grown in culture. Where these large amounts of material are needed, growth of the parasites in rats is sometimes preferred. To achieve statistical validity for our experiments and to complete the experiments for which we have received substantial external funding, we anticipate using up to 4200 mice and 80 rats over 5 years of study.

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?

Trypanosome infections can be fatal in rodents, death being preceded by progression through a predictable series of symptoms on a relatively predictable timescale. [REDACTED] so that experimental outcomes can acheived without the infection leading to illness or death, which is a rare occurence (less than 5% of infections) . Mice and rats are monitored for their disease progression based on a numerical scoring system and undue suffering prevented by humane killing should the infection progress to a level where death his anticipated within a few hours. As a consequence, the overall severity for our experiments is classed as moderate. At the end of experiments animals are euthanized and parasites are harvested.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We routinely grow trypanosomes in cell culture. However, their life cycle development does not progress normally in cell culture, such that the production of stumpy forms is best achieved in parasites grown in mice. Furthermore, the evaluation of the effects of disrupting particular parasite molecules and processes can only be accurately determined by studying parasites growing in the natural context of a bloodstream infection in a mammalian host. For the isolation of large numbers of parasites, the ability to harvest tens of millions of parasites from a single infection makes some experimental approaches feasible that would not be possible using cultured parasites that are in an unatural growth medium and isolated from the host (and thereby in the absence of an immune response and where molecular signals are not at natural levels due to their non-physiological accumulation or turnover in culture). Furthermore, parasites grown in culture do not generate synchronous populations of stumpy forms that are required to analyse molecular events in the population that refelct molecular events going on in each individual parasite. Coinfections also require to be studied in the context of a functional immune system. Whilst interactions between parasites in culture can be informative, ultimate validation of any effects observed needs to carried out using parasites growing in a mammalian host where the combination of immunity, signal production and turnover can contribute to the infection dynamic.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Trypanosome growth in mice progresses on a relatively predictable course, such that it is straightforward to generate reproducible and statistically valid datasets that satisfy external scientific scrutiny (i.e. where the probability of the observed outcome being incorrect is less than 5%). We are experienced in analysing and predicting parasite virulence in mice and so can minimise distress whilst deriving the necessary scientific information from infections. Genetic manipulations of trypanosomes often involve use of gene silencing or gene overexpression techniques, which are controllable using chemicals supplied in the rodent drinking water (e.g. doxycyline). This provides well controlled analyses because phenotypic comparisons between induced and uninduced populations provide a robust experimental outcome using the same parasite material. [REDACTED]

Refinement

Explain the choice of animals 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.

Refinement

Mice are used to monitor trypanosome infections in laboratories worldwide. This allows comparisons of the infection profile and kinetics of proliferation and differentiation between studies and between laboratories. The symptoms linked to trypanosome infection in mice are also predictable allowing us to track the progression of infections with a scoring system allowing defined humane end-points. A staged approach will be adopted when novel drugs are used.

PROJECT 208

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Functional analysis of muscle genes in mice
Key Words	heart, skeletal muscle, Popeye genes, mutation, striated muscle disease
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The POPDC genes encode a family of membrane proteins, which mediates adrenergic signalling in the heart. Recently, a number of patients were found, which developed heart and muscle disease carrying point mutations in POPDC genes. We want to define the fundamental functions of PODPC genes but also learn how these genes are involved in causing heart and skeletal muscle disease. This research will therefore provide novel insight into heart and skeletal muscle disease mechanisms, which provide novel opportunities to develop novel therapeutics.

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)?

Defining the network and pathways in which POPDC proteins are acting will provide fundamental insight into the processes maintaining structure and function of striated muscle. Insight into the disease mechanisms associated with mutations in POPDC genes causing striated muscle disease will be prerequisite for the development of novel therapies.

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

We will be using mice that are genetically modified to study the function of genes in the heart and skeletal muscle. Over the period of 5 years a total of 10,800 mice will be used.

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

We will generate and breed mouse mutants such as POPDC mutants and study their development of cardiac and skeletal muscle dysfunction. POPDC mice will develop over time an irregular heart beat and muscle weakness. It is also known that mutants display spontaneous phases of respiratory distress, particularly under stress. Animals will be carefully monitored using a traffic light system defining levels of severity (from green to red). Animals which have reached red alert level will be euthanized by Schedule 1 method. The breeding of POPDC mice is a moderate procedure. Transgenic animals will be phenotypically assessed with the help of an ECG analysis. For this purpose, telemetric ECG devices will be implanted into transgenic mice in order to be able to monitor heart rate and ECG in the conscious animal. This experimental approach allows for example to study the cardiac response to physical activity. This procedure is moderate and no major adverse effects are expected. Careful surgical technique and aseptic procedure will be performed, which normally prevent any complications and all animals will receive antibiotics and pain killer to minimize pain. After the end of the experiments animals will be euthanised by a Schedule 1 method. The ECG analysis of POPDC mice is a moderate procedure. In another experiment, animals will be injected with cardiotoxin, which causes muscle necrosis and is an established model to study muscle regeneration. This experiment will cause transiently some pain, which will be monitored using the grimace scale system (https://www.nc3rs.org.uk/mouse-grimace-scale). After the end of the experiments, animals will be euthanised by a Schedule 1 method. The regeneration experiment using POPDC mice is a moderate procedure. In order to introduce DNA constructs into skeletal muscle, the foot muscle (musculus flexor digitorum brevis) will be injected with DNA constructs and electroporated. No surgical intervention is required. The muscle is only injected twice and subsequently the electrodes will be applied for electroporation. This experimental protocol is the most refined method of gene transfer into muscle. The injections of DNA into the footpads and the subsequent electroporation do not have noticeable adverse effects on the animals. After the end of the procedure, mice are able to amble normally around the cage. Pain killer will be given as an additional precaution. At the end of the experiments, animals will be euthanised by a Schedule 1 method. The electroporation experiment is a moderate procedure.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Cardiac and striated muscle disease can only partially be modelled using cell culture models. However, wherever possible we will be using cell cultures or computer modelling. However, in vitro experiments cannot fully substitute research in

animals. Thus, at present, animal models cannot be substituted. All mice will be housed in groups where possible, with appropriate environmental enrichment and fed according to current institutional 'best practice'.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We keep up with developments in the field to avoid duplication of experiments and use knowledge derived from complementary technologies (including our work in cell culture, *Xenopus* oocytes and the zebrafish model).

We shall only use a minimum number of animals in our experiments. Breeding strategies are performed by qualified personnel and aimed to avoid unnecessary generation of animals. Statistical analysis will be applied to estimate minimum numbers of animals required for valid comparisons. Power calculations are used to project numbers.

Results will be published according to the ARRIVE Guidelines.

Refinement

Explain the choice of animals 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.

Refinement

The mouse is an excellent model organism to study the genetic basis of cardiovascular and skeletal muscle disease. The mouse POPDC mutants develop skeletal muscle or heart disease, which is intended and the basis of our studies and possibly accompanied by weight loss and stress sensitivity. With the help of a traffic light system and the grimace scale, the health status of the animals will be closely monitored. When animals develop severe phenotypes (red alert), they will be used immediately or sacrificed. Experiments in the zebrafish and in cell culture models will be complementing our efforts in the mouse. However, neither zebrafish nor cell culture models can fully substitute our experimental work in mice. The mouse has the advantage that its heart resembles the human heart in many aspects and also has the great advantage of extensive possibilities of genetic manipulation and being an established model in cardiac physiology. The use of Cre-mediated tissue- and time controlled ablation of POPDC genes in mice will allow to limit the loss of gene function to a single organ or tissue, which will limit the overall phenotype severity and at the same time will be extremely informative with regard to the tissue-specific functions of POPDC genes and the cell autonomy of the phenotype in mutants.

PROJECT 209

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanisms of Organogenesis
Key Words	human syndrome, Birth defect, Cancer
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

People with Van Maldergem syndrome have multiple abnormalities including defects in their brain, face, limbs, kidney and bones. Van Maldergem syndrome is due to mutation in two proteins known as Fat4 and Dachsous. Van Maldergem syndrome shows that these proteins are critical for human development but very little is known about how these proteins function. Our goal is to increase our understanding of how these defects arise and to determine other potential roles of Fat4 and Dachsous1 during human development. We also want to understand how loss of these proteins may contribute to post-natal disorders and disease.

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

Our research will reveal the potential functions of Fat4 and Dachsous1 during human development. We will ultimately use this knowledge to help repair damaged organs in both Van Maldergem syndrome and other related syndromes/disease through tissue engineering.

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

We have mouse models that mimic the human syndrome and we will use these. Within the project, up to 1252 genetically altered animals will be used per year and up to 6260 during the course of the project.

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

The majority of this proposal will use post-natal mice that show no phenotype and appear normal. For one study, we will analyse growth of the facial bones and in this study, there will be altered facial growth which could affect feeding; these mice will

be closely monitored to identify signs of distress and will be culled before weaning. All mice used any this licence will be culled humanely at the end of the study by the appropriate method which will minimise stress/suffering.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

An organ develops by precisely controlled interactions between different cell types within that organ. Each organ has a specific shape e.g. kidney versus lung and different cell types arranged in precise pattern. We do not know all the cell and growth factor interactions that are involved in organ development and also can not currently control the spatial arrangement of different cell types in a dish to mimic organ development. Therefore, we currently have to use animal models. However, when we have identified the specific cell types that Dchs1 and Fat4 function in we plan to pursue research with cell lines where we can.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Usage of animals will be maximised by co-ordinating research within the research group who investigate distinct aspects of development (e.g. craniofacial and musculoskeletal), and with collaborators investigating other organs, to ensure that all tissues of interest are collected and utilised to the maximum efficiency and productivity. Routinely, tissue is also kept so we can carry out analysis of multiple tissues from one mouse.

Refinement

Explain the choice of animals 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.

Refinement

We are using mouse models as mice are genetically very similar to humans, their organs develop in a similar manner, and there is significant evidence that they can be used a models for human disease. Mice can also be manipulated in more sophisticated ways than other vertebrates allowing precise dissection of the role that genes play in organ development and function. We should be able to use our results

in mice to predict gene function in humans. We know this pathway plays key functions in the development of several organ systems and we predict we will be able to use this knowledge to understand human disease ultimately leading to the development of therapeutics for human diseases.

All experiments will be designed to minimize any potential stress on the animals and mice will be continuously monitored and assessed to determine their health and disease status to prevent stress and any severe organ deterioration.

PROJECT 210

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Bioservices support
Key Words	Disease prevention
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This Project Licence aims to provide the scientists with specific biological materials and micro organism-free animals as required in order to conduct the work permitted under their own Project Licence.

For example, in the investigation of disease and the development of vaccines and other beneficial interventions, occasionally scientists may require small volumes of normal blood for purposes other than those defined in their own Project Licences. Additionally, some completely lab-based scientists may not have a Project Licence as they do not work in the whole animal. This may be allowed under the conditions of this licence provided the cost/benefit analysis for the procedure is deemed to be of sufficient merit by the AWERB and the procedure does not exceed the Home Office guidance on minimal severity protocols.

Scientists whose Project Licences cover the procedures they will perform on SPF or Gnotobiotic animals usually do not have the caesarian section on their own Project Licence.

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)?

Understanding immune responses greatly aids in the development of vaccines and other interventions that can help prevent or minimise disease and thus improve animal health, welfare and productivity. There are animal diseases that are difficult to diagnose and prevent and there are emerging diseases, new diseases of livestock which science needs to understand in order to treat and prevent.

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

This Project Licence would allow a number of mostly farmed species but also some "laboratory" species of animals to be kept specifically for providing blood samples, or for the delivery by Caesarian section under general anaesthetic of infection-free offspring. Over a 5 year period, the number of animals kept under this Licence should not exceed 20 cattle, 310 sheep, 50 pigs, 26 rabbits, 12 rats, 100 mice and 80 birds.

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?

All procedures on the licence are of minimal severity and no adverse effects are expected, with the exception of the Caesarian-derived, disease organism-free offspring: in this case the donor dam is euthanased at the end of the surgery without regaining consciousness. The animals of farmed species are kept in a natural 'farm' environment indoors or outdoors, in company, and the majority will be used to provide small blood samples at specified, relatively infrequent, intervals. Laboratory animals are kept indoors in clean environments designed to provide the Five Freedoms as far as possible and in compliance with Home Office requirements. Other than mild discomfort associated with needle-prick there should be no adverse effects and the Severity is Mild. At the end of a specified limited time and following veterinary examination, some of these animals may go on to another experiment under a different licence, but this requires Home Office approval. Some animals are humanely euthanased for reasons of age or prevailing circumstances. Animals that have been kept on our disease research station cannot go into the food chain or be moved on to another premises for reasons of Biosecurity.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Blood can only be obtained from the actual animal. The subsequent scientific work is generally lab-based. Blood may be used to grow pathogens, replacing the infection of the live animal.

Live neonatal animals in the numbers required can only presently be obtained from an adult female animal.

By publishing and sharing findings, materials and methods, the scientific community endeavours to use alternatives to live animals wherever possible.

Currently, different species are required on this licence depending on the proposed use of the blood products e.g avian blood is required for haemagglutination-inhibition assays.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

SPF and Gnotobiotic caesarian derived animals:

All scientific work using this Licence has to be approved by the AWERB which includes a statistician. This ensures that experiments are well-designed and use the minimum number of animals required to get valid results. It also co-ordinates use of animals and procedures to prevent duplication.

Using ultrasound where possible, dams with high numbers of offspring are selected for neonatal delivery, reducing the number of dams required.

Provision of blood:

The main purpose of this Licence is to obviate the need for different research groups to keep their own animals for sourcing blood from a particular species, thereby reducing the total number of animals required.

Numbers of animals required will be minimised by re-using donors for subsequent samples, keeping to limits defined in the licence.

Refinement

Explain the choice of animals 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.

Refinement

The staff taking the blood samples have many years experience of this procedure in farmed and laboratory species. In sheep, the ventral neck area is kept shorn which makes the procedure easier. A 'Combi Crate' (a type of crush suitable for sheep) has been purchased and is used to augment restraint as necessary. In general, the blood donors become accustomed to the minor procedures and experience minimal stress, so rarely struggle and can be restrained with minimal physical effort by an experienced handler.

[REDACTED]

PROJECT 211

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Understanding Thymus Function and Immune Reconstitution
Key Words	Immune system, T cell development, Thymus, Autoimmunity, Immune reconstitution
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The thymus is an essential organ of the immune system. The primary role of the thymus is to support the development of T cells that are act to provide immune protection against infection and tumour formation. In addition, the thymus plays a vital role in preventing the emergence and activity of T cells that would otherwise drive autoimmune disease. Despite the critical role of the thymus in ensuring protective immunity, the basic cellular and molecular interactions that control thymus development and function remain unclear. This project aims to investigate the identity of the cells and molecules that control T cell development within the thymus and understand how these impact upon T cell-dependent immune protection. These studies will inform further work where we will seek to use identified regulators of thymus function to enhance thymus activity and T cell driven immune protection in settings where this is defective.

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)?

Continued output of T cells from the thymus is required to establish and maintain a peripheral T cell pool of sufficient size and diversity necessary to provide effective protective immunity. In addition, correct thymus function is required to delete those T cells capable of inducing autoimmune disease. Thymus function however can be disrupted in a variety of settings, including: inherited genetic deficiencies, chronic age-associated loss of thymus tissue (thymus atrophy) and acute loss of thymus tissue in response to damage e.g. irradiation or infection. In all instances, loss of thymus activity leads to reduced T cell development and a corresponding increase in susceptibility to infection and potential for tumour formation. This project seeks to advance our fundamental scientific knowledge of the cells and molecules that control

the development, maintenance and loss of thymus tissues. The identification of novel regulators of thymus function will provide potential routes that may allow manipulation of T cell development with the ultimate longterm goal of improving health and wellbeing through improved immune protection.

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

Approximately 18,000 mice will be required to breed and maintain colonies of mice, and perform the planned experiments over the five year duration of the project

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

The majority of experiments will reach a sub-threshold level of severity which will involve the generation and breeding of genetically-modified mice for the isolation of tissues after they have been humanely killed. In some cases however, a moderate level of severity will be reached due to the bone marrow and tissue transplantation approaches used to precisely identify the cell types and molecules involved in thymus function. To assess the cells and signals involved in either loss or recovery of thymus tissues some animals may be subjected to low dose irradiation in order to induce acute (transient) loss of thymus and T cell development. Following loss and recovery of thymus tissues, it will be necessary to assess the function of the T cells generated by testing their ability to provide protective immunity. In some cases the T cells that develop may possess the potential to drive autoimmune disease when thymus function is defective. In all cases, animals will be carefully assessed and any suffering will be kept to a minimum. All mice will be killed humanely at the end of the protocol or should pre-defined humane end points be reached prior to the end of the protocol. In the course of these experiments, animals will also necessarily be subjected to injections, blood sampling and/or modification of their diet. Any adverse effects to animals will minimised by ensuring the rigorous use of the most refined approaches by skilled staff

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

This project requires animals as the study of thymus function and T cell development must be examined in live animals due to the complex dynamics and cellular interactions involved. For example a key part of T cell development is not only the development of such cells in the thymus, but their subsequent export into the blood circulatory system and entry to peripheral tissues to provide immune protection. At

present no in vitro experimental systems exist that allow this to be accurately reproduced in non-animal models

We will conduct continued review of the scientific literature in order to identify any newly emerging models that may provide the opportunity to replace existing animal models wherever possible.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Rigorous experimental design and use of statistical analyses will be performed to ensure that we are able to accurately and robustly generate meaningful experimental outputs whilst ensuring the minimum usage of animal numbers. We have extensive experience of breeding and using mouse models to investigate thymus function, and have published extensively in high-impact scientific journals. We will ensure that we use such existing experience and experimental frameworks to ensure the use of minimum numbers of animals. Further, in order to reduce the numbers of live animals undergoing experiments, we will use in vitro culture of thymus tissue within a petri dish to identify candidate molecules capable of influencing T cell development prior to performing experiments in live animals. Using such systems will help ensure that non-functional candidates do not progress to live animal models thereby keeping the number of animals undergoing such experiments to a minimum.

Refinement

Explain the choice of animals 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.

Refinement

The mouse replicates the placental pattern of human development in a short (21 days) gestation time and provides an accurate model of the development of the human immune system, including thymus. It is also the only species that provides a range of natural and induced mutants with defined genetic alterations that allow the study of target molecules crucial to the function of the immune system. A large body of published data has been generated characterising the immune system in mouse models, and the vast majority of knowledge regarding thymus development and function is based on the use of murine models. Using genetically-modified mice we are able to precisely identify the role of specific cells and molecules and define their role in thymus function. The approaches described are established and every effort has been made to develop refined techniques causing minimal adverse side effects. An example of specific refinements include the development of new tissue

transplantation approaches that involve the placing donor tissue under the skin of the ear. Such techniques can potentially reduce the use of more invasive transplantation approaches and may aid in reducing the risk of infection

PROJECT 212

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The physiological roles of phosphatidylinositol 3-kinases
Key Words	Therapeutics, immunology, Translational, cancer, metabolism
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We study signalling pathways, which is the processing of information within cells, tissues and animals. When these systems go wrong, this can lead to disease.

We have created mice with altered genes, called mutant mice, to study signalling pathways and assess if these mice develop diseases (for example malformed vessels) or are protected from some diseases (for example are protected from allergic stimuli). Ultimately, we wish to develop drugs that can alleviate human disease and to further our scientific understanding of the signalling pathways involved.

In order to achieve these goals mutant mice are initially created, followed by a full screening to find out if these mice have any abnormal pathology, behaviour or physiology. In some instances, we can challenge these mice, for example give them an allergic stimulus, to see if the inactivation of the gene protects. In this way, we can identify genes against which pharma can make drugs to treat human disease. In previous work, our studies have instigated and informed drug development, and human clinical trials using these drugs are currently in progress in cancer and allergy, with one drug approved for cancer therapy in 2014.

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 knowledge gained from this licence will not only contribute to the understanding of how signalling pathways operate, and when disrupted, cause disease, but also to develop therapeutics for these diseases. So far we have found that disrupting the pathways of the PI3K gene family may result in, or protect diseases of metabolism, immunology and cancer. Therefore therapeutics will be developed to help alleviate

these disorders. As a result of our work and those of collaborators, drugs have been developed that are in phase II clinical trials and are helping to treat people with cancer.

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

Mice, 3000 per year Rats, 150 per year

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

Animals will be required for this project as many of the disease we study, such as allergy and cancer are very complex and cannot be replicated in cell or tissue systems. Also, for drug-based studies, the whole mouse is required to elucidate any side effects that may occur, which would not show up in cells or tissue systems. For these protocols, the likely severity bands will vary from mild to moderate. In the case of collecting organs or cells for tissue culture work, the severity reached would be sub-threshold for non-harmful mutant strains. Procedures will consist of humanely killing mutant mice, for cell-based tissues and blood for pathological analysis. Other experiments would consist of injection of cells that cause cancer or the injection of substances that cause allergies and to administer medicines to see if these diseases get better. Also, minor surgery, such as the removal of tumours may also be carried out. Adverse effects likely to be encountered would be weight loss or gain, subdued behaviour, and hunched posture. Adverse effects will be monitored for daily and mice will be culled before the humane endpoint is reached or before if sufficient scientific data has been collected. Protocols have been designed such that mice are subjected to minimal suffering. Also, pilot studies will be initially carried out on very small numbers, to give an indication of what scientific data would be generated and the degree of suffering imposed, such that subsequent experiments can be refined to minimise suffering. At the end of the experiment, animals will be killed and tissues used for in vitro studies or biochemical analysis.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animals will be required for this project as many of the diseases we study, such as allergy and cancer are very complex and cannot be replicated in cell or tissue systems. Also, for drug-based studies, the whole mouse is required to elucidate any off target effects that may occur, which would not show up in cells or tissue systems.

Mice are good models to study signalling pathways, as they resemble humans closely; anatomically, biochemically, physiologically etc.

Before animals are used, cells from tissue banks will be cultured in order to first characterise whether the signalling pathways we wish to study are affected in diseases such as allergy and cancer and whether drugs can restore these pathways to normal functioning.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We propose only to work with mice and rats. Statistical analysis will be used in order to keep the minimum number of mice required to make a valid scientific conclusion. We plan not to use more than 3000 mice per year for all our experiments and for collaborators specialised in other disciplines, such as behaviour and imaging. We are also developing tissue culture based systems such as making organoids - miniture organs in culture - which will help replace the need to do procedures in mice.

Refinement

Explain the choice of animals 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.

Refinement

Protocols have been designed such that mice are subjected to minimal suffering. No mouse would be subjected to more than a moderate amount of suffering. Also, pilot studies will be initially carried out on very small numbers, to give an indication of what scientific data would be generated and the degree of suffering imposed, such that subsequent experiments can be refined to minimise suffering. The latest methodology for scientific procedures will be adhered to which causes the least amount of harm to the animals.

PROJECT 213

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Decoding brain activity during cognition, sleep and disease
Key Words	Cognition, Sleep, Psychiatry
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project centres on three interrelated questions about brain function: (1) How do our brains balance flexibility with stability? (2) Why is brain activity during sleep organised into such strikingly distinct patterns? (3) How is brain activity impacted by genetic variations that increase risk for psychiatric disorders like schizophrenia?

(1) Our world is always being updated. Second by second, we keep track of the constantly changing barrage of information reaching our senses, scanning the scene for anything that may prove important. On the other hand, we must avoid becoming overwhelmed by all this change, forming stable perceptions and memories that mean we can recognise the world around us. How do brains achieve this balance? Are precisely the same groups of brain cells ('neurons') that respond to a given stimulus or event then reactivated when we re-encounter that event? Or does redundancy in the system mean different subsets of neurons can step in, allowing flexibility without compromising stability? These questions will be addressed by monitoring the activity of large numbers of neurons simultaneously in rodents learning and updating information about their world, mapping patterns of brain activity to behavioural outcomes (e.g. learning to find chocolate in new locations).

(2) In rodents and humans alike, sleep is central to brain health. "Off-line" brain activity during sleep helps to process newly-learned information, filtering out unimportant details and joining the dots between experiences that share common features. It is likely that sleep is therefore part of the answer to question (1), yet we know relatively little about why sleep is structured into alternating cycles of REM (rapid eye movement) and non-REM stages. This project will track how learning new information during wakefulness drives changes in brain activity during subsequent sleep.

(3) Understanding how the brain works remains one of the greatest biomedical challenges, not least because it is a necessary step towards treating the many brain disorders that ruin people's lives. The biggest gaps in our understanding of brain function lie in the middle ground between molecular mechanisms and behaviour. For example, we have a list of at least 108 genetic variations that can increase risk of schizophrenia, and the clinical symptoms of schizophrenia have been documented in great detail; yet we do not know the mechanisms linking genetic variants to neuronal activity. By first establishing how brain activity stably encodes information about the world, then mapping how that brain activity changes in rodents genetically engineered to carry schizophrenia risk genes, this project will begin to assess how brain activity during wake and sleep is altered by genetic factors that increase risk of psychiatric disorders like schizophrenia.

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)?

If successful, these experiments will: - Shed light on how brains adapt to their surroundings to let our past shape our future. Until we know whether neurons – individually or in groups – are activated in the same patterns repeatedly over multiple days, we cannot understand the fundamentals of how brain activity gives rise to healthy (or pathological) behaviour. - Explain why sleep is structured into cycles of different stages. We spend approximately one third of our lives asleep, yet we currently do not know precisely what or how non-REM and REM sleep stages contribute to our understanding of the world. This is particularly important in light of the prevalence of sleep disorders in modern society (20-30% of adults report sleep problems). - Pave the way for rational design of new treatments for psychiatric disorders.

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

Approximately 360 mice and 580 rats over 5 years.

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

These studies will not work unless the rats and mice we work with are healthy and content – otherwise they will not perform behavioural tests or sleep normally. Housing and handling conditions are therefore designed to optimise welfare and any surgical procedures make use of full anaesthesia, health monitoring and analgesic regimens recommended by veterinarians. Animals may sometimes have controlled access to food to motivate task performance, but they will get to enjoy highly palatable and nutritious rewards during behavioural tests that tap into to rodents' natural preferences (e.g. exploring mazes, poking their noses in holes). This range of experiments will cause mild to moderate discomfort. Once experiments are

complete, animals will be humanely killed and brain tissue collected to support further data analyses.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The only way to map the route from brain activity behaviour is to record brain activity during behaviour; this requires the use of behaving humans or animals. [REDACTED] Similarly, wherever possible we marry work in behaving animals with in vitro studies of neural activity (e.g. in brain slices) and with computational models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The methods we use are highly efficient at gathering large volumes of data from individual animals using a within-subject design, meaning the number of individual rats and mice used is kept to the absolute minimum required to allow statistically robust conclusions. Each experiment is planned carefully, with each animal assigned a 'Study Plan' to optimise its contributions to the dataset, typically addressing multiple objectives during a single procedure (for example by recording during both behaviour and sleep).

Refinement

Explain the choice of animals 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.

Refinement

Most of the experiments will be performed in rats, a choice backed up by decades of research into their neurobiology and behaviour. The brain regions we study are organised and connected similarly in rats and humans, and rat sleep architecture cycles through non-REM and REM stages as ours does. We can make specific genetic manipulations in rats using – and validating – technology that could translate to genetic engineering to treat human disease. Rats are also large enough to surgically implant with devices for recording brain activity over multiple days; the technological advances driven by these experiments (e.g. wireless recording so animals do not need to be tethered) refine methods for monitoring brain activity, and

also complement similar advances applicable to humans. Finally, the data analysis programs used in this project are also state-of-the-art, complementary to those required to analyse human EEG and brain imaging data, and are made freely available online.

PROJECT 214

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Regulation of platelets by CD148 and G6b-B
Key Words	Platelets, signalling, proteins, bleeding, clotting
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We study proteins, such as CD148 and G6b-B, on the surface of blood cells, called platelets. Defects or alterations in these proteins can change the way platelets regulate the balance between excessive blood clotting and bleeding. Despite the important roles played by these proteins in maintaining human health, for example in heart disease and stroke, it is scientifically not well understood how they control platelet function.

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)?

Platelets play a vital role in controlling bleeding and blood clotting. However, it remains unclear how platelet function is regulated. Increased platelet activation is associated with diseases caused by blood clots, such as heart attack and stroke. Current anti-platelet therapies are effective at preventing the formation of life-threatening blood clots; however, they have severe bleeding side-effects. Thus, there is an urgent need for the development of improved anti-platelet drugs that reduce the risk of clot formation, but have no associated bleeding side-effects. This objective can only be achieved through a comprehensive understanding of the molecular mechanisms controlling platelet activation and function, from which novel drug targets will be identified. A drug that can prevent blood clotting without leading to excessive bleeding will save lives. As a secondary benefit, by better understanding how the number of platelets in the circulation is controlled we can develop ways of culturing platelets outside of the body and treating a variety of human platelet disorders. This will also reduce the need for mouse models to study platelet biology.

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

Over 5 years, we would expect to use no more than 27,500 animals in total – 10,000 animals for scientific protocols and 17,500 to breed the genetically altered strains required.

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?

In this project, the majority of mice will have blood taken under terminal anaesthesia. Occasionally some mice will be given modulators of platelet activity or cell numbers by injection or orally.To avoid repeated injection of modulators of platelet activity in a few cases surgical minipumps may be implanted under the skin to supply the substance. We expect only transient mild to moderate adverse effects following surgery, which will be alleviated by giving pain killers. There may, however, be an increased risk of spontaneous bleeding in some of these mice, although from our previous experience this does not happen. In addition, we have never observed hypothermia as a result of these treatments. If animals show signs of distress like weight loss between 10% and 20% and staring coat they will be closely monitored, but if the animals show intermitted hunched appearance, irregular breathing patterns or reduced activity they will be humanely killed to avoid further suffering. Genetically modified mice experiencing clinical signs of disease will be used in experiments before the animals wellbeing is affected significantly, i.e. conditional Shp1KO mice will be used before swollen feet impair gait and movement. When new pharmacological substances are tested, the dose finding will be done in pilot experiments on a small number of wild type mice to limit the number of mice which might be experiencing adverse effects, animals will be carefully monitored and humanely killed if the adverse effects exceed moderate severity.Some mice may also undergo procedures to test the bleeding and clotting functions of their platelets. These procedures will all be performed under terminal anaesthesia throughout the experiments and will therefore experience very little suffering or discomfort. Finally, some mice will also be exposed to limited doses of radiation to partially deplete their own platelets and thereby allow us to efficiently introduce platelets with altered function. High doses of radiation can cause sickness in mice therefore we will use doses that are well tolerated. However, any animal showing signs of distress/pain reaching a moderate severity limit will be killed to avoid further suffering

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

No *in vitro* techniques are currently available that can fully replicate platelets and platelet function. Physiological platelet turnover experiments can only be performed

in whole animals. Human platelets will be used in experimental setups that do not require genetically altered platelets.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will work according to the ARRIVE guidelines and use the NC3R experimental design assistant software as well as statistical analyses will be used to ensure that the minimum numbers of mice are used in order to obtain scientifically sound data. Breeding strategies will be optimised to generate the required number of genetically altered mice with the least number of animals used in breeding protocolsIn addition, multiple tissues/organs (e.g. blood, spleens, bone marrow) will be collected from each mouse to maximise data collected. State-of-the-art equipment with increased sensitivity, requiring small amounts of platelet samples, will be used to investigate platelet biochemistry and function, circumventing the need to pool platelets form multiple mice.

Refinement

Explain the choice of animals 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.

Refinement

The blood clotting systems of humans and mice are very similar, utilizing the same blood proteins and cell types. Mice are therefore used extensively to study and understand human disease mechanisms of thrombosis and haemostasis. The mouse has been selected, because they are well established and reliable experimental models, there is an extensive literature validating mice as models of human physiology and disease, and many reagents, including antibodies and inhibitors, are currently available to study platelet function in mice. For these reasons, mice are the gold standard for studying platelet biology and physiology. The majority of procedures outlined in this project will be under terminal anaesthesia, and therefore harm to the animals will be low. Mice that receive platelets and platelet modifiers or mice with uncharacterised genetic mutations will be closely monitored. The advice of the named veterinary surgeon and named animal care and welfare officers will be taken to ensure animal suffering is minimised where possible.

PROJECT 215

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Immunology and immunopathology of leishmaniasis
Key Words	Leishmaniasis, Neglected Tropical Diseases, Immunology, Vaccines, 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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We are developing vaccines and new medicines to combat a parasitic disease called leishmaniasis. Leishmaniasis is transmitted by the bite of an infected sand fly, and is caused by different species of single-cell parasites called *Leishmania*. 350 million people are at risk of contracting leishmaniasis in 98 countries worldwide, and ~1.5 million new cases and 20,000 - 40,000 deaths are reported annually. Leishmaniasis in dogs is also a major veterinary problem in Europe and South America. No vaccines are currently available for use in man, and most current medicines either have side effects, do not work in all parts of the world or are too expensive for general use in lower and middle income countries.

In the most serious form of leishmaniasis, the parasites spread from the skin to the internal organs. It is not known how this occurs, but our immune response to the parasites causes severe tissue damage. It is also not known how these parasites are picked up by sand flies in order for the disease to be transmitted and whether drugs and vaccines can affect this process. To answer these questions, we need to first examine the natural history of the disease and then how it is modified by intervention. For both practical and ethical reasons, such studies are not possible in man, so we must make use of experimental models of infection. Our studies use mice, which develop a similar but less severe disease than humans. Infected mice have normal behaviour and for the most part do not outwardly look “ill”. We continually explore new ways to study this disease e.g. using advanced imaging techniques, using in vitro models and using computational models. However, these are not yet sufficient to predict the efficacy of a new vaccine or new medicine or evaluate their safety.

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 programme has three aims: First, to identify new vaccines and medicines. Many of the new medicines we will examine are already in use for other human diseases, which limits the number of pilot studies we need to perform and makes further development and application simpler and more cost effective. We have made significant progress in making a therapeutic vaccine for leishmaniasis and have just started the first trial in patients with one form of persistent disease. However, further experiments in mice will be required e.g. to evaluate safety and efficacy in combination with other medicines, to test this and other vaccines for the prevention of this and other forms of leishmaniasis and to find out if other diseases that people may have will affect how the vaccines and drugs work. If our vaccines and drugs studies are successful, this will have a major impact on the health of millions of people worldwide. Second, we use mice to maintain colonies of sandflies, the vector that transmits leishmaniasis. This allows us to understand how parasites develop in flies and what makes them infective to man. We also use these flies to test whether our vaccines can block disease transmission. Understanding more about how these parasites are transmitted will also help others develop new strategies for disease control to reduce the burden of leishmaniasis in communities. Third, we are studying the parasite to understand why disease is different in different parts of the world and to help develop vaccines and drugs that will work globally. These studies involve genetically manipulating the parasite and then assessing whether we have changed its ability to cause disease or to be transmitted. Initial assessment is performed in vitro, but mice are required to evaluate how the changes we make affect disease progression.

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

All of our studies are conducted in adult laboratory mice. Most of these have normal immune systems, but some have been bred to have defects in immunity. This helps us understand how our vaccines and drugs work and also is useful to model certain co-infections e.g. HIV that suppress immunity. We expect to use approximately 4000 mice over the next 5 years for this research.

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?

Mice infected with parasites that cause visceral leishmaniasis look outwardly healthy. Some mice (approx. 10%) may progress to develop signs of illness. Mice infected with Leishmania causing skin disease develop sores which may ulcerate. In some studies, where we need to understand how different infections (e.g. malaria) might affect vaccination, mice will have clinical disease associated with both infections. At the end of all infection experiments, mice are humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The immune system is complex and we do not yet have the tools to reliably predict how vaccines and drugs work in humans without using animal models. Data from such models are also currently required by regulatory authorities before we can proceed to clinical trials.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We use the minimum number of mice to achieve statistically meaningful results through application of good experimental design and the use of approaches that allow maximum data capture from each individual animal e.g. using non-invasive imaging for longitudinal studies of disease progression. Data from all our animal research are used to help construct computational models of how the immune system works and interacts with drugs. These models are helpful for making decisions about which combinations of drugs might need to be tested in animals.

Refinement

Explain the choice of animals 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.

Refinement

Leishmaniasis is a complex group of diseases and no animal model captures every aspect of human disease. The mouse provides the best experimental tool for understanding how our immune system operates and for testing early stage vaccine and drug development. Once we have identified likely candidates for further study, our approach has been to conduct the minimal number of animal studies as required to satisfy regulatory authorities and allow rapid translation into human trials. All animal research is conducted in facilities that provide the best standards of husbandry and care. Many of our animal studies examine early time points before disease fully develops, reducing welfare issues even further.

PROJECT 216

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The role of Seminal Fluid Proteins (SFPs) and reproductive microbiota in fertilisation dynamics
Key Words	Seminal fluid proteins; reproductive microbiota; fertility; poultry.
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

1) Determine how proteins naturally present in the seminal fluid of male red junglefowl affect fertility and the behaviour of females following insemination.

2) Study the microbes living within the reproductive tract of males and females and determine how they may influence sperm quality in red junglefowl.

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)?

A more comprehensive understanding of reproductive biology, as well as livestock production and health.

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

Approximately 350 male and 350 female red junglefowl will be studied over the course 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 levels of severity? What will happen to the animals at the end?

Healthy adult males will be exposed to semen collection through abdominal massage and sampling of cloacal microbes through gentle wipes of the cloaca. Some of these males will also exposed to semen collection by mating with a female under controlled conditions (staged mating) and/or blood collection. Healthy adult females will be exposed to microbial sampling through gentle wiping of their cloaca. Some of these females will be exposed to artificial insemination and to mating with males under controlled conditions (staged mating), and a subset of these females

will also be exposed to blood collection and/or feeding of edible dyes to mark the yolk of their eggs. Birds will reach the end of the protocol at the age of 7 years old, which is when they will be terminated to prevent them from suffering adverse effects of advanced age.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Whenever possible, we have replaced live animals with studies of biological samples in the lab (i.e. *in vitro*). The focus of the project is vertebrate reproductive biology, and there is no opportunity to conduct the other parts of the project using any other than conscious animals because the complexities of the female reproductive tract in internal fertilizing vertebrates rule out *in vitro* approaches. This is the only animal model where routine semen collection through staged matings is feasible. In a tame population of red junglefowl, we have identified the animal model that guarantee the success of the project while minimising animal welfare problems.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have minimised the number of animals required, in three major ways: 1. *In vitro* assays (see above); 2. Synergistic sample collection will enable us to sample fewer birds and fewer times by using the same samples for both Aims of the project simultaneously; and 3. Study design and experimental procedures have been refined e.g. through blinding, randomisation, and use of adequate controls. Continued use of individual birds will enable a more powerful experimental design based on intra-individual variation.

Refinement

Explain the choice of animals 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.

Refinement

As the wild ancestor of the domestic chicken, the red junglefowl offers a unique opportunity to study a natural (i.e. not domesticated or artificially bred) organism that is simultaneously relevant to domestic poultry for which we can harness the power of

research tools developed for domestic chickens. We have refined our methodology to minimise the severity of the procedure: e.g. by exposing harnessed females to males in controlled mating we drastically reduce the amount of sexual harassment that females can suffer in nature.

PROJECT 217

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanistic basis of information processing in neural circuits
Key Words	Synapse, Neuron, Circuit, Behaviour, Learning
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Correctly interpreting sensory information (e.g. touch, visual scenes and sounds) and generating coordinated movements are essential for our survival and normal behaviour. Indeed, deficits in these functions, which are common hallmarks of neurological disorders (e.g. schizophrenia, ataxia and autism), have debilitating effects. However, surprisingly little is known about how neural circuits represent, distinguish and learn sensory sequences or how sensory information is disentangled from self-generated input arising from movements. The overall aim of this research is to discover how the synaptic and cellular properties of circuits enable the brain to perform these functions.

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 benefit of this research programme is to generate new scientific knowledge about how neural circuits in the brain represent, transform and learn sensory information and coordinate movement. Developing a better understanding of the physiological mechanisms underlying normal brain function will also provide a basis for understanding how genetic and disease-induced changes in proteins, synapses and neurons cause aberrant network behaviour during neurological disorders, which are currently poorly understood and impose a heavy burden on society. The data collected during this research programme will also be used to build, refine and test biologically accurate models of brain circuits. A lasting benefit of this animal research will be to provide computer models that can be used by other scientists in their future research. Such models consolidate quantitative knowledge, can generate new hypotheses through prediction and enable neuroscientists to develop a better understanding of complex neural systems. An additional benefit of such models is

that they can lead to more informed/defined hypotheses, thereby enabling greater refinement in experimental design and reduced animal usage.

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

This 5-year program of scientific investigation into brain function will involve a team of 15 scientists. The breeding of multiple genetically modified mice strains accounts for the vast majority of animals used due to only a fraction of the progeny inheriting the transgene. These together with the mice that will be used for behavioural experiments and mice/rats for tissue preparation will total approximately 1200 p.a.

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

The protocols involve procedures of mild or moderate severity. Animals typically undergo short surgeries under general anaesthesia where we make very small windows in the skull so that we can image and record the activity of neurons. Potential adverse effects include postoperative stress and discomfort, but this will be minimised with analgesia. When unexpected clinical signs appear, we will immediately consult our designated welfare officers and vets. At the end of each procedure animals will either be euthanised according to schedule 1 or another humane method, under terminal anaesthesia and the tissue will be utilized for ex vivo experiments.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Experiments on live brain tissue and intact animals are essential for gaining new knowledge on the properties of synapses, neurons and networks and there is no equivalent or alternative to using animals. Cell culture is not suitable because synaptic, neuronal and network properties all change in the cell culture preparation. Moreover, investigation of how information about the body and surroundings is represented and processed requires that animals are intact. Wherever possible we use computer models to test possible scenarios before experiments are carried out, but it is essential to test model predictions experimentally for them to have scientific value.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The minimum number of animals will be used to achieve the aims of the experiments and ensure reliable and reproducible results. The data generated in this project will largely be differences in neuronal and circuit responses following a perturbation, sensory stimuli or a defined behavioural task. Each experiment is designed, as far as possible, to include its own control, reducing variability, increasing statistical sensitivity and thus minimizing the number of animals required to reach statistical significance. Variability across animals will be minimized by using well defined strains of mice, with little genetic variation. We also apply methods of experimental design which enable us to estimate the minimum number of animals required to detect an effect, where possible. We will be conducting our experiments to be able to publish according to the ARRIVE guidelines.

We use and develop methods and experimental techniques that maximise the data collection from the minimum number of animals. We will micromanage the breeding of mice to ensure that the number of animals bred matches as closely as possible the number required for experiments. Cryopreservation of transgenic lines is utilised whenever there is a sustained gap in experimental use in order to minimise breeding surplus. Lastly, for acute slices, tissue from an animal is shared between researchers whenever possible.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the least sentient spices that are appropriate for this type of study. The basic synaptic, neuronal and network properties found in mice are common to all mammals and makes them a good model system for studying human brain function. Moreover, the large range of genetically modified mice available are critical for labelling specific neuronal subtypes with fluorescent indicators that report neuronal activity and optogenetic transducers that can activate and silence neurons with light.

All surgery will be performed using appropriate anaesthetics/analgesic regimes to minimise pain. Surgery will only be performed by fully trained and competency assessed staff with procedures under regular review to identify further refinements to minimise animal suffering.

Mice will receive environmental enrichment and will be group housed wherever possible.

PROJECT 218

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Pathogenesis and control of chlamydial infections in livestock
Key Words	Chlamydia, Vaccination, Pathogenesis, Livestock
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aim of this project is a better understanding of the infectious causes of reproductive loss and failure in farm livestock, as a result of bacterial infection with the organism Chlamydia abortus that is responsible for the disease known as Enzootic Abortion of Ewes (EAE). These bacteria are known to cause infertility, abortion, and the birth of weak offspring and have a significant impact on the health and welfare of sheep, goats, cattle and pigs in the UK, as well as having major economic effects on the global farming industry. Moreover, there is a risk to human health, where some of these pathogens are known to be associated with severe disease in pregnant women as well as loss of the fetus.

The project will endeavour to (i) improve methods for the detection and diagnosis of chlamydial infections in farm livestock; (ii) achieve a greater understanding of the pathogenesis of chlamydial infections in farm livestock; (iii) identify key components of protective EAE vaccines; and (iv) evaluate and develop new novel vaccine formulations for controlling chlamydial infections in farm livestock.

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 overall outcomes of the outlined project will lead to improvements in the management of the diseases caused by the bacterial organism Chlamydia abortus, including better identification of infected animals and better methods for controlling infection, which in turn will result in improved animal health and welfare, a reduction of the environmental transmission of infections from animal to animal, a reduction in the potential for zoonotic transmission of chlamydial infections to humans, as well as

reduced green-house gas emissions resulting from greater farming efficiency and productivity.

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

We expect to conduct 3-4 vaccine efficacy trials over the course of the 5 year licence, utilising approximately 700 sheep. Approximately 600 fertile hens' eggs will be required to produce infectious material to 'challenge' the animals and assess protective efficacy of the trial vaccines.

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?

Challenge or infection of ruminants with Chlamydia may result in a mild transient fever and/or lameness at the injection site which is not expected to last more than 2 days. Infection of pregnant animals with Chlamydia abortus will result in abortion or the birth of weak offspring in a proportion of the animals (usually 40-60%) with the mother largely unaffected. Any offspring deemed to be too weak to survive or suffering will be euthanised immediately. Administration of novel vaccines is not expected to cause any adverse effects but may result in mild irritation and discomfort at the injection site. The protocols used in these experiments will ensure at all times the health and welfare of the animals being used. All animals will be closely monitored and assessed and end-point criteria have been identified based on clinical presentation and symptoms to ensure that no animal will suffer. Any animal deemed to be in distress or suffering will be humanely killed. Severity has been assessed as being moderate taking into account all of these factors. Animals will be euthanised at the end of the experiment. Growth of Chlamydia in embryonic avian eggs will result in death of the fetus and is assessed as being of moderate severity. Any surviving fertile eggs are not kept beyond 18 days of gestation and are humanely killed by chilling at 4C. Animals which have received an unlicensed vaccine in the form of a novel vaccine are humanely killed and are not allowed to enter the food chain.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

A complete biological system is frequently required to study the course of clinical disease and the whole body response to infection. The mechanisms of bacterial transmission from one animal to the next and disease interventions such as vaccination cannot be studied in non-animal alternatives. Animals are only used after in vitro alternatives have been thoroughly investigated and exhausted, or that the

use of alternatives will likely mean that the outputs will not be achievable, which would then result in experiments being repeated and require a greater number of animals being used.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Reduction measures include the design of animal studies to maximise collection of biological materials/data from each study. Alternative strategies have been developed to produce the vaccine material that is tested to reduce the number of fertile hens' eggs required.
Use of a statistically valid minimum number of animals per study will be determined by previous experience and/or power analysis.

Refinement

Explain the choice of animals 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.

Refinement

The species chosen are those for which the disease is most relevant in the field i.e. farm livestock. Pilot experiments will refine protocols – antigen, dose, regimen and adjuvant required to establish an optimised vaccine. They also provide data that allows the severity of disease to be minimised by using established animal models with humane end-points.

All animals will be closely monitored and assessed and end-point criteria have been identified based on clinical presentation and symptoms to ensure that no animal will suffer. [REDACTED]

PROJECT 219

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Studying how signalling proteins control development and regeneration in zebrafish
Key Words	Zebrafish, Signalling, Development, Ageing
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Our aim is to learn more about the cellular processes occurring in embryogenesis and during regeneration. Currently we have a very limited understanding of how signal proteins control cell differentiation at a cellular and genetic level. In particular, we want to know how Wnt and Shh signals control cell differentiation during complex tissue formation in early embryogenesis. In addition, we then want to understand the roles played by these signalling genes during neural regeneration and regeneration of the zebrafish fin. We will identify new targets of these pathways to learn more about regeneration and wound repair in vertebrates.

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

We envisage that our programme of work will lead to improved understanding of the genetic and cellular processes involved in cell differentiation and cell migration leading to organ formation in embryogenesis. We will elucidate the beneficial functions of Wnt and Shh signalling during regeneration. Furthermore, we will screen chemicals interfering with this signalling pathways to identify treatments that could be used to promote wound healing in vertebrates including humans.

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

We will use zebrafish for the whole of the project. We anticipate using a maximum of 11000 over the 5 years of the project, many of these will be breeding colonies of zebrafish that carry fluorescent reporter proteins, which are not expected to experience any suffering or ill effects. We will undertake regular systematic reviews to ensure animals cannot replaced.

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

The types of adverse effects that we expect to see for the larval fish might include alterations to bone formation. We will carefully monitor these fish to ensure that they can swim even if their skeletal shapes are abnormal. Occasionally when we are using a new drug or generating a new genetic modification a small number of larval fish may develop adverse effects such as heart oedema or brain malformation, these fish will be killed as soon as these defects are identified. For adult and ageing fish we expect the adverse effects to be during regeneration that resemble human wound healing. These would be likely to prevents formation of epithelial appendages, which results in scarring of the epidermis in fish. We will monitor the behaviour of the fish daily and any fish that can no longer maintain their position in the water will be killed under terminal anaesthesia. By carefully monitoring fish to ensure that that they do not exceed these end points we expect these studies to be of mild severity.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The fin is a complex 3-dimensional structure containing many different cell types and which is subject to frequent movement, as such there are very few non-animal models that exist and none that model all aspects of cell behaviour in the fin. We therefore need animals to understand what happens to the different cell types as a model for common human disorders such as wound healing. We will seek, review and incorporate tissue culture assays to replace animal experiments throughout the project duration if appropriate.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will continue to develop 3D in vitro models and computational models that allow us to model the effects of altered cell signalling and migration during fin regeneration, and through using these we expect to reduce the numbers of zebrafish larvae used by around 25%. For those experiments that require animals we perform power calculations to define the minimum numbers required to achieve defined levels of statistical significance and we consult with statisticians and other biologists when planning experiments. Efficient breeding practices will be incorporated to

reduce animal numbers. We will use the NC3R’s research design tool which feeds into the ARRIVE guidelines.

Refinement

Explain the choice of animals 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.

Refinement

We have chosen the zebrafish as most refined animal model available and complement our studies with complex tissue culture experiments. By using the translucent zebrafish in which we express fluorescent proteins in cells of interest we can non-invasively watch many cellular processes in the fin, allowing us to collect dynamic data with minimal surgical intervention.

To minimise suffering and discomfort the fish will be monitored daily and when there is any concern advice will be sought from the named veterinary surgeon and/or the Named Animal Care Welfare Officer and appropriate action taken.

The responsible team members, i.e. the PI and the technician, have more than a decade of experience and expertise in using zebrafish as a model organism in aquatic research facilities in the UK, the US and in Germany.

[REDACTED]Regulations to ensure animal health and minimise the risk of infection through environmental management are in place.

PROJECT 220

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Improving vaccine-induced mucosal immunity
Key Words	Vaccine, Mucosal, Formulations, Sexually Transmitted Infections (STIs)
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project aims to develop vaccine candidates that generate protective immune responses against diseases that typically enter the body through mucosal tissues and skin surfaces. We further aim to develop the vaccine in a manner that is non-invasive and female controlled and that has the capacity to be retained on skin mucosal surfaces. This retention on skin mucosal surfaces may improve the immunogenicity of our formulations. Our ideal vaccine would be one given systemically by intramuscular injection with boosting inoculations given mucosally at the site where potent vaccine generated immune responses would be most effective.

It is not clear how best to generate protective immune responses against invading microbes in mucosal tissue, particularly the vagina, that are long-lasting and that may prevent infection. We know that it is possible to protect against vaginal mucosal infection with for example, a virulent immunodeficiency virus, but there are a number of complicating factors that make these vaccinations difficult. There is rapid leakage from the site of administration, degradation of vaccine components by enzymes in mucosal secretions and differences in immune responses dependent upon the stage of the menstrual cycle. In humans we can easily deliver vaccines at a particular stage of the menstrual cycle but we need vaccine formulations that can resist leakage from the vaginal vault and the degradative action of enzymes and that can target those cells within the mucosal tissue which can start an immune reaction.

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)?

Achieving the aim of designing and developing a vaccine that is able to generate immune responses that are targeted to the vaginal, nasal or rectal mucosal tissue

would have a fundamental impact on both human and veterinary health. Many pathogens enter the body at the mucosal portal of entry and being able to fight the invading microbe at that point would present the best chance of preventing disease, particularly for those viruses and bacteria that are able to evade the immune system once they have entered the body (Tuberculosis and HIV being excellent examples). Having sterilising mucosal barrier immunity would prevent a contagious disease from spreading freely throughout a susceptible population, limiting the health impact of diseases with high transmissibility (Flu, Ebola etc). Each formulation we test has the potential to become a candidate vaccine for human trials. We believe our study has the potential to produce either a new vaccine candidate or to improve current vaccines and so can have significant benefit to people.

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

The animals we will use in our study are primarily inbred mice as we have a range of assays that can accurately measure their response to vaccination, allowing us to comprehensively examine immunity, significantly reducing the number of animals. All of our studies involve injection (brief minimal pain) or surface application of our vaccine formulations in a similar manner to human vaccination and it is crucially important to us that the animals are not injured/distressed by the administration of our vaccines as distressed animals will not respond immunologically in the same way as healthy unstressed animals. We strive to use the fewest animals possible in our experiments and have calculated how many are needed based on statistical validity. We use many more mice than other species and over the five year period we would expect to use approximately 700 mice per year or 3500 mice over the licence period. We would expect to use approximately 100 rats per year over the period of the licence (500 total) and less rabbits with 50 being required per year (250 total) or guinea pigs with 60 being required per year (300 total).

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?

As our project focuses on vaccination processes we expect and aim to have very few, if any, adverse effects. In our previous project licence we had no adverse effects that were attributable to the vaccine formulations or the administration of them to the animals. We also saw a rapid return to normal activity after any procedure – whether it was an injection or blood sampling or imaging, where they received a gaseous anaesthetic. We only use a gaseous anaesthetic when necessary to prevent the animal from feeling pain (the brief electroporation technique) or to prevent them moving (imaging). It is critical to us that our vaccines are translatable to humans and therefore any significant adverse effects are an extremely negative outcome. Both the injections, the formulation and the method of administration must be as well tolerated as possible, both in the animals and ultimately in humans. It would not be in our interest to have a vaccine or delivery

methodology that had significant adverse effects. In all cases the animals that have undergone any procedure will be assessed immediately after the procedure for any adverse effects and then again within 3 hours of the procedure and again at 24 hours after the procedure. This is in addition to the general husbandry observations and assessment of the animals. When we infect animals that have received a vaccination we would expect to observe adverse effects. The influenza challenge model causes weight loss and illness in the mice and we have set our experimental endpoints to ensure the animals would be culled well before their suffering became severe. We have classed the infections to be a moderate severity protocol and we have a number of criteria by which we would measure and closely monitor the progression of the influenza infection. The chlamydia infection has virtually no effect on the health of the animal with only slight temporary weight loss. 'We will actively communicate with and seek advice from NACWO/NVS as required. LASA (Laboratory Animal Science Association) guidelines will be followed in all aspects of the care and use of the animals. Data will be published in accordance with ARRIVE principles to maximise the information published while minimising unnecessary studies. If the animals do become ill we will administer additional care in the form of wet food. If animals suffer from common husbandry issues, such as excessive barbering, or tail dermatitis we will seek the advice and care of the NACWO/NVS and potentially separate the mice and in the case of dermatitis administer emollient cream. In all cases, the animals will be killed at the end of the experimental procedures.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We need to use animals for our studies to determine the *in vivo* efficacy of our vaccines as *in vitro* assays are unable to replace experimental animals as they do not adequately model the immune system and cannot provide information on the generation and development of immunity after vaccination.

The events and process that follow immunization are extremely complex and involve recruitment of cells and activation followed by relocation of stimulated cells to specialized immune structures where further maturation occurs. The specific microenvironments where these cells encounter the vaccine antigen and then undergo differentiation and maturation are impossible to replicate outside of a living animal.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Each of our vaccine formulations go through extensive pre-animal testing to exclude any formulation with toxic effects on cultured cells or that has no capacity to reach immune cells within mucosal tissue explants. We can measure the capacity of our vaccine formulations to target these immune cells in various cell culture studies and in small scale pilot animal experiments to determine if we have sufficient reason to do definitive experiments in animals. Therefore any vaccine formulation and delivery method must pass a number of ex vivo hurdles before we would even consider their use in animals, reducing the number of animals used. We have also carefully calculated, based on our work in a previous project licence the minimum number of animals required to be certain that our measurements are meaningful. This is extremely important as these careful calculations ensure that experiments will not need to be unnecessarily repeated to allow us to determine if a difference between a placebo and experimental group is real and significant. Experiments will be designed and results will be published in accordance with ARRIVE principles to maximise the information published while minimising unnecessary studies.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the lowest vertebrate group available to us that have a fully functional immune system that is similar to that in man. These inbred animals have a highly reduced variability in genetic background and will enhance the probability of achieving a statistically significant result with the minimal number of animals required to fulfil the conditions for significance. We would use rats in circumstances where larger volumes of sera are required to perform the assays. Mice have a non-functional TLR8 pseudogene and are unresponsive to a number of TLR8 agonists. Guinea pigs are the animal of choice for a number of viral infections such as influenza, CMV and herpes. We would use Guinea pigs when intending to either confirm in a second animal model the efficacy of a vaccine regimen or when mice are not amenable to certain viral infections. Rabbits are larger vertebrates that can provide much larger volumes of serum and mucosal wash samples for analysis. The vagina of the rabbit is significantly less stratified than that of the mouse and will not require hormone thinning treatment. Furthermore, rabbits produce antibodies with more flexible structure to mice allowing better neutralization responses to HIV antigens (in this respect rabbits are more similar to humans than mice). Mice, unlike rabbits, have a very high background of non-specific neutralizing activity in the

serum making analysis of specific antibodies problematic. Rabbits are used as the gold standard test for vaginal irritation, their use will expedite which formulation to take into preclinical GcLP toxicology studies.

We will minimise the suffering of the animals in several ways. Our experimental vaccination protocols are of a mild severity level and we are highly experienced in handling, manipulating and sampling all of the above species. Our infection challenges will be closely monitored and have humane endpoints that prevent escalation of severity limit beyond the moderate level.

PROJECT 221

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Regulation of antiviral immune responses
Key Words	Virus, Disease, Immune system, Vaccination, Immunotherapy
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The aims of this project are to improve our understanding of 1) how inflammation induced by viruses can be treated, 2) how cells of the immune system can be harnessed to improve control of virus infections and 3) how the immune response induced by viruses can be manipulated to improve protective immune responses induced by virus-based vaccines.

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)?

These findings will assist 1) the design of immune-based therapeutic strategies to treat the immune-mediated damage triggered by viral infection and 2) vaccination strategies that use viruses as the basis of their vectors.

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

Protocol 1: 2500 mice Protocol 2: 500 mice Protocol 3: 2000 mice Protocol 4: 2500 mice Protocol 5: 100 avian eggs

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

Animals used in experimental procedures will be handled frequently (likely daily) during the first week of infection. This will involve injections and other forms of administration, and monitoring of virus-induced weight loss and illness. Through good handling techniques, distress caused to the animal from being restrained will be minimised in terms of time and discomfort (a single animal will typically be restrained for less than 30 seconds). Mice may also be handled at regular intervals

throughout the remainder of the experiment where they may be injected and monitored. Should virus induced weight loss reach maximum permissible levels, animals will be killed by a schedule 1 method. Based on working with mouse models of viral infection since 2004, it is expected that for the majority of animals used in this project, the severity level will not exceed “Mild”. At the end of experiments, mice will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Viral pathogenesis is the result of a complex series of interactions between viruses, the host cells in which they grow and the immune system that responds to the infection, which itself is a complex highly-regulated series of interactions between different cells and molecules. Furthermore, assessment the possible efficacy of vaccines and cellular therapies also involves complex assessment of the interactions of viruses and the host immune system. Thus, animal models are necessary as there is currently no way to recreate these complex interactions *in vitro.*

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Our extensive experience in these viral infection models will allow us to accurately design experiments that use the minimum number of mice to achieve statistically significant results. We have developed methodology in our lab to assess virus replication and inflammation by non-invasive saliva and blood sampling, respectively. These assays will be used in many studies to minimize mouse numbers required.

Further, our experience in these models provides us with critical insight into the kinetics of virus-induced immune responses and disease, enabling us to study defined time-points that represent the critical times of infection. Also developments in our lab using cutting-edge methodology enable us to obtain vast amounts of information from small samples, maximising the information that we can obtain from each mouse.

Refinement

Explain the choice of animals 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.

Refinement

The mouse provides an excellent model in which to study the immune system. Mice are well characterised immunologically, and their immune systems to viruses closely resemble those of humans. Infections of viruses and administration of reagents involve minimal handling of the animals and our experience in these models enables us to study mediators of inflammation without requiring the induction of a severe infection.

Mice will be monitored frequently be weight loss measurement and mice that are suffering from overt weight loss will be humanely killed by schedule 1 methods.

PROJECT 222

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Targeting cellular signalling and calcium handling in models of cardiovascular disease
Key Words	cardiovascular disease, ageing, inflammation, cardiotoxicity
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Heart failure remains a leading cause of death worldwide. Statistics are not helped by the fact that current medication for heart disease, although effective in the short-term, can have detrimental effects in the longer-term due to the non-specific nature of the drugs involved. There is therefore a pressing need for better medication in order to improve patient outcome. Heart disease and ultimately heart failure occurs as a result of physical or chemical stress being placed on the vessels that carry the blood around the body and on the heart itself. Sources of stress include (i) underlying disease in the vessels and heart, (ii) ageing of the vessels and heart or (iii) toxicity of certain types of medication to the heart e.g. medication used to treat cancer. We still do not fully understand the process by which these sources of stress result in the cells of the blood vessels and heart adapting and behaving abnormally. In order to improve heart medication in the longer term, we need to have confidence that drugs will specifically target the culprit(s) within cells of the heart and blood vessels that contribute most significantly to development of heart failure. Importantly, we can use animal models of cardiovascular disease as well as cardiotoxicity to very effectively introduce these same stressors and investigate in detail the changes that occur in the blood vessels and heart. [REDACTED]The key aims of this project are (i) to identify changes in specific protein function that may occur in parallel across different cells of the blood vessels and heart following disease or ageing and demonstrate how this impacts upon heart function (ii) to identify key proteins (e.g. CaMKIId) in cells of the blood vessels and heart that contribute to the toxic effects of drugs (e.g. anti-cancer drugs) on cardiovascular function and (iii) to examine whether disrupting key proteins (e.g. CaMKIId) at a functional level can reduce disease progression or toxic effects on the heart.

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 short-term this project will advance understanding across the cardiovascular and oncology community of scientists and clinicians. In improving our understanding of common mechanisms that become defective in the heart and blood vessels following different types of stress, we can advance drug design towards more selective targeting across different groups of patients. This could have implications for patients suffering from cardiovascular disease as a result of progressive atherosclerosis and/or hypertension as well as patients who may have developed heart problems as a result of taking a particular medication e.g. medication for cancer. Ultimately, this will result in improved patient care for those suffering from heart disease as well as cancer.

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

Mice and rats will be used. Maximum numbers would total 1700 (~1200 mice and 500 rats) over 5 years.

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

Adverse effects on the animals in the models of heart disease we propose to use will be rare. From experience, any adverse effects are likely to occur during surgery when there is a risk of bleeding (<20%), and only very rarely (<1%) following surgery. The number of animals undergoing surgery will be limited. Animals could exhibit breathing difficulties while under anaesthesia for ultrasound however since we use inhalation anaesthesia, this can be well controlled. The level of severity for these procedures is moderate. For animals that are genetically manipulated the level of severity is mild. All animals will be euthanised at the end of the procedure.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

For studies investigating cardiovascular disease, it is imperative that we use adult animals to recapitulate what happens in the adult human. We cannot use neonatal cardiac cells or cell lines for this work since they are completely different to the cells that exist in the adult heart and blood vessels. In order to understand how certain stresses affect the heart, we need to be able to monitor effects on the cardiovascular system in the whole animal as well as at a cellular level. This is particularly important

since ultimately, we wish to investigate how interfering with particular targets in the cell may impact upon heart function in the live animal.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will ensure wherever possible that one animal is used for both heart and blood vessel work so that multiple outputs can be achieved without the use of multiple animals. For cell-based work, we can maintain cells in culture over a period of several weeks. This will allow for repeat experiments without the need for repeat animal use. For tissue-based work where possible we will process one heart or vessel in multiple ways to ensure we can use the tissue from one animal for multiple types of assay. Importantly, by sharing animals through the ShARM initiative or indeed in-house, one animal can be used for multiple research purposes. Where possible, and in particular for research on ageing, we will aim to use the heart and/or aorta from animals that are already being used by other research groups. This ensures that optimal use is being made of one animal with little waste.

Refinement

Explain the choice of animals 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.

Refinement

Mice will be used for both surgical and pharmacological models of disease. We already have a significant amount of data from a mouse model of disease so it makes sense that future work in this area is continued using the same species. This also ensures that future work on genetically-modified animals can be performed. Both models of disease are minimally invasive to the animals but allow a significant degree of heart remodelling to occur to allow us to investigate early stages of heart dysfunction. Ultimately, as indicated previously, we would plan to refine our approach for inducing cardiac remodelling by switching from a surgical approach to a pharmacological approach. We will use rats for studies on ageing and cardiotoxicity because we can gain more material from rat hearts and blood vessels and this will allow more cost-effective projects using fewer animals. All animals will be housed in our Biological Procedures Unit and will be individually housed when required to provide optimum care (e.g. when recovering from surgical intervention). They will be closely monitored by staff and students within our research group and by staff within the unit.

PROJECT 223

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Understanding the role of the host immune response and tumour microenvironment to enable the generation of novel immunotherapeutic strategies to treat cancer
Key Words	Immunotherapy, microenvironment, cancer, antibody, host immunity
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Cancer is the cause of one in four of all deaths in the UK with a greater proportion still affected by cancer during their lifetime. Despite advances in screening and diagnosis cancer incidence continues to rise, largely as a result of an aging population and the impact of our modern lifestyle. The majority of cancers are diagnosed late and treatment frequently involves surgery, accompanied by chemotherapy or radiotherapy. Despite these invasive and toxic interventions patients often relapse due to the survival of small numbers of tumour cells. In spite of decades of work on treatment regimens, the survival for many cancers remained unchanged until very recently. Clearly there is a need to develop new therapies for use either as an alternative to or in combination with conventional treatments. Antibody immunotherapy, whereby the power of the patient's own immune system is harnessed to eradicate cancer cells, is an attractive adjunct to current treatments as it offers the possibility to be administered with maximal specificity, minimal toxicity and provide long term immune protection against recurrence.

The overall aim of this project is to understand the role of the host immune response and tumour microenvironment in cancer to enable the generation of novel immunotherapeutic strategies. The specific objectives are:

1) Determine the mechanism of action of antibody drugs, how they may be potentiated by pharmacological drugs and/or immunomodulatory agents/and or other modalities with the aim of developing improved clinical reagents;

2) Understand how the immune environment both systemically and locally changes and develops with cancer and the potential impact that this may have on anti-cancer immunotherapy;

3) Elucidate the mechanistic requirements for and then develop strategies to overcome cancer induced immune suppression thereby promoting immune responses to cancer using mAb, mAb derivatives and/or other immune-modulatory agents.

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)?

Immunotherapy has the potential to provide long-lasting curative protection from tumour relapse in patients. The results from our work are intended to be directly translatable to clinical application and patient benefit. The principles obtained from our work will also inform the fields of clinical infection, autoimmunity, transplantation and allergy as well as veterinary science.

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

Animals will be used in experiments between July 2017 and June 2022. On the basis of our current research, it is estimated that we will use approximately 16,000 animals during this 5 year period. We will apply for amendment to the licence if monitoring shows that this is likely to change significantly.

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

The vast majority of the experiments will result in no adverse effects. When using tumour models, mice will be culled at the humane endpoint. In some cases the administration of immunomodulatory substances may cause transient adverse effects, but these will remain within the moderate severity limit, otherwise the mice will be culled.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We are committed to replacing mice where possible and we evaluate immunotherapeutic agents on cell lines in vitro when we can. However, immune modulating agents act upon multiple cell types across the body concurrently and this cannot be adequately modelled in vitro at the current time. Similarly, to study the

interactions between an ongoing immune response and a growing tumour, or to evaluate immune-mediated pathology there is unfortunately no viable alternative to in vivo modelling using animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Mice used across experiments are inbred thereby minimising intra-group variability and allowing reduced mouse numbers for experiments. Experiments are always designed with the fewest animals consistent with obtaining statistically valid results. We have performed Power analysis to determine the numbers of mice required to deliver statistically significant results, although through experience we find we can often use smaller numbers of animals without sacrificing statistical significance. Where appropriate, small pilot experiments are carried out where simple factors such as dose or route of administration are not clear. Where multiple inter-relating parameters are to be evaluated, larger factorial experiments are performed to prevent use of excess mice as controls. In recent years significant technological advances have enabled more information to be obtained from one individual mouse than was previously possible (e.g. using multi-parameter flow cytometry and micro-array technology), enabling multiple parameters to be assessed simultaneously from small samples. These technologies thereby facilitate longitudinal studies and reduce the need to cull multiple mice at different time points to sample from the spleen for instance; we aim to fully exploit these new techniques fully where possible. Tumour cells will be stored frozen when possible to prevent mice being used to passage tumour in vivo.

Refinement

Explain the choice of animals 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.

Refinement

Mice are the least sentient mammal species with an immune system similar to humans. Mice represent a relevant animal model for these studies and the clinical successes now being reported using immunomodulatory drugs against cancer were dependent on data arising from such murine studies. Numerous mouse cancers have been studied and the availability of genetically altered strains, and commercially available reagents aids this research. Environmental enrichment, good husbandry and frequent monitoring ensure high welfare standards. Few adverse effects are anticipated but, should any occur, rapid steps will be taken to ameliorate them or humanely cull affected animals.

Death is not an acceptable end-point for cancer models: we have established end-points for humane culling before pain/distress occurs, based on accepted guidelines. Many tumour lines develop as subcutaneous nodules, allowing easy monitoring of tumour size. However, the visible or palpable size of the tumour is only one of the criteria used for determination of humane endpoint. Experiments will therefore be terminated before tumour size limits behaviours (feeding, drinking, movement) or before or at the first signs of, tumour associated symptoms or poor condition of the animal according to well defined guidelines (e.g. facial expression scales; www.nc3rs.org.uk/assessment-pain-using-facial-expressions-laboratory-mice-rats-rabbits-and-macaques). Occasionally, following therapy a subcutaneous tumour resolves from the inside out giving the appearance of ulceration; we have adopted a scoring system from Lloyd and Wolfensohn in the Handbook or Laboratory Animal Welfare and Management to ensure that these are managed with minimum adverse effects to the mice. While the maximum severity limit for much of the work to be conducted under this PPL is set as 'moderate', through experience and good management of the mice, we have found under our existing PPL that the actual severity of most experiments is 'mild'.

PROJECT 224

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Mechanisms of mammalian birth defects
Key Words	neural tube defects, birth defects, inborn metabolic disorders
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project uses genetically altered mouse strains to determine the processes in the embryo that predispose to birth defects. The work will: (i) identify the key genes, (ii) determine the embryonic processes, and (iii) discover new methods for prevention. We aim to reduce birth defects by 'correcting' development in the womb or after birth, as appropriate.

Among the genes that predispose to birth defects such as neural tube defects, the same genes are also involved in inborn metabolic disease such as Non-Ketotic Hyperglycinemia (NKH) for which is there is currently no cure. The mouse model for NKH that we have developed provides the opportunity to better understand underlying mechanisms and to test novel therapy.

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)?

Clinical unknown and need for research. Birth defects affect 1 in 40 pregnancies worldwide and, represent a huge burden on society. Children with birth defects are often handicapped, physically, mentally, and require life-long medical treatment. However, medical science is unable to prevent most birth defects, and new information in this area is urgently needed. This project particularly focuses on neural tube defects, including spina bifida and anencephaly, which affect approximately 1 per 1,000 pregnancies worldwide. NKH is a rare genetic disease that causes profound developmental delay and early death in affected children. Current treatment is not effective and there is no cure, although this project could lead to novel treatments in future.

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

Project plan. Breeding colonies of genetically altered mouse strains are used to reveal how birth defects arise. Each strain is bred for: (i) genetic studies to localise the key genes, and (ii) to produce embryos for studies of how birth defects develop. In some studies, embryos growing in a test tube are supplied with nutrients to determine whether spina bifida can be prevented. Mice that are studied for post-natal rescue of NKH are maintained to 12 weeks of age. Around 8000 mice per year will be used overall for breeding and mating to produce embryos and experimental offspring for our research. Around 300 rats per year are killed to produce serum for the embryo cultures.

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?

To keep suffering and animal numbers to a minimum. E, early embryos are mainly studied, before pain sensation has arisen. Embryos can be grown in a test tube, to minimise the number of pregnant mice used. When studies of pregnant females or juveniles/adults are essential, we use non-surgical approaches: for example, feeding a nutrient-deficient diet. The majority of the work will be under the mild level of severity. Pregnant mice will be culled for collection of embryos. Mice that are maintained to post-natal stages will either be used in breeding or will be analysed for experimental outcome, with collection of tissue and blood at the end of the experiment.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Why animals have to be used. This research addresses the complex question of how embryos develop before birth, and what can go wrong leading to birth defects. This can only be studied properly in whole developing embryos. Our ultimate goal is to understand the mechanisms of human embryo development as a contribution to understanding the causes and possible prevention of birth defects. However, experimental analysis of human embryos during early stages of development is not practical or ethical. Instead, we will use a mammalian model, the mouse, in order to gain information of maximum applicability to humans. Nevertheless, for some studies we replace the use of live animals by examining human embryos, using tissue culture cells and developing computer simulations.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Experimental design, analysis and reporting will conform to the NC3Rs' ARRIVE guidelines. Breeding schemes and experimental protocols will be designed to ensure use of minimum numbers of mice necessary to generate data with sufficient numbers for fully powered statistical analysis.

Embryos can be grown in a test tube, to minimise the number of pregnant mice used. Rats are killed to produce serum for the embryo cultures. We have developed methods to allow 50:50 dilution of rat serum with serum-free media. This allows a reduction in the number of rats used in this research.

Refinement

Explain the choice of animals 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.

Refinement

Mouse and human embryos undergo a closely regulated developmental process and by using mice we can benefit from the array of genetic and molecular tools available as well as access embryos for imaging. For example, we use mouse genetic models with defects in the same genes as cause the equivalent conditions in humans. Organ development in lower vertebrates such as fish often does not occur by the same mechanisms as in mammals.

Early embryos are mainly studied, before pain sensation has arisen. When studies of pregnant females or juveniles/adults are essential, we mainly use non-surgical approaches: for example, feeding a nutrient-deficient diet.

PROJECT 225

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Signalling mechanisms in vertebrate development
Key Words	birth defects, head, skull, regeneration, genetics
Expected duration of the project	5 year(s) 0 months

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

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We are studying how genes control development of the head. We wish to learn about normal development and how the process goes wrong, leading to problems that affect newborns and cause serious disfigurement, such as cleft palate , small jaws and premature fusion of the skull bones (craniosynostosis). We use animals where we can alter their genes and examine the head. The majority of our studies involve cells from animals that are “normal” or genetically altered. We also study the offspring of mutant animals, allowing us to assess multiple tissue types *in vivo*, such as brain tissue adjacent to skull bones.

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)?

Results from this study will advance our knowledge of how the human head develops. From this, we hope to be able to diagnose disorders quickly and with time, develop methods of prevention or therapy. Together, this work will allow us to identify factors which can accelerate repair and regeneration of the cranial skeleton. At present it is very difficult to rebuild or replace the damaged head skeleton, with many patients needing repeat surgeries. Our studies in animals will allow us to reliably test new therapies. These studies will serve as proof-of-principle experiments prior to translating these therapies to humans. The long-term goal will be to reduce or replace invasive surgeries in patients.

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

The experiments are designed such that samples obtained from genetically altered and control animals are used several different ways; for example, tissues are analyzed for growth rates as well as changes in gene expression. This increases the power of the experiments and reduces the number of procedures needed. We

anticipate using approximately 2500 mice, 500 frogs and 200 chickens over the course of five years. The majority of animals are used for breeding and are not stressed.

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?

In general, we are using these animals for breeding purposes, which are natural and therefore should cause no adverse effects. Because of this, most of our protocols are of mild severity. In some rare experiments we administer compounds that alter development or allow us to see cells in a microscope. Such agents may be given in food or water, or by injection. Administration of compounds is also routine, and should cause no adverse effects; however, animals will be monitored for discomfort and will be killed by a Schedule 1 method if discomfort becomes evident. In a few cases, we allow administration of agent by inserting a mini-pump; this is a surgical procedure, under anaesthesia, and animals are monitored during recovery. In some cases, we may introduce a wound in bone. This is performed under anaesthesia and analgesia and animals are monitored for stress. Because these are surgical protocols involving general anaesthesia, they are of moderate severity; however, in our experience, animals do not experience adverse effects. Nevertheless, animals will be closely monitored for any discomfort. The frogs used are Xenopus, which live for many years and lay eggs with a single hormone injection. The treatments cause them no harm and frogs rest between treatments. Chickens used are common farm chickens and laying eggs causes no stress. For all three species, most analysis occurs in the embryonic stages before the nervous system is functional. In our experience these animals experience minimal discomfort and we expect all Xenopus and chicken procedures to be of mild severity. Animals will be killed at the end of the protocol. Any animal will be immediately killed by Schedule 1 method if it shows evidence of suffering that is greater than minor and transient or in any compromises its normal behaviour.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animal models provide a great deal of information on the causes and progression of birth defects. In particular, genetic mutants often phenocopy human conditions and lead us toward molecular events underlying developmental processes. These kinds of studies need to be performed in vertebrates where analogous genes are implicated in the development, healing and regeneration of analogous structures. For example, the distinct bones that comprise the human skull vault are equivalent to the

bones in the skull of the mouse, not just in function but also in embryonic origin and molecular identity. Furthermore, the three-dimensional development of the human skull is greatly influenced by surrounding tissues, such as the underlying brain and meninges. While we can test interactions in a limited way in a tissue culture dish, or by computer simulations, we still need to return to animal models to truly test our hypotheses.

In some cases we can replace mouse experiments with embryonated chicken eggs, which provide an excellent model for the study of early developmental stages, with eggs being readily available and developing independently outside of the uterus. This allows easy accessibility for molecular manipulations and easy monitoring of welfare.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The mouse is an incredibly powerful system for understanding mammalian biology, particularly with the availability of genetic models. To reduce mouse numbers used, we make use of explant and tissue cultures systems which are routine in the lab. This makes efficient use of genetically modified mouse lines as a single embryo can contribute multiple samples and conditions, reducing numbers. Co-culture and 3D organoid methods can also allow us to examine tissue-tissue interactions maximising the use of each animal while ensuring minimal animal numbers.

To minimize use of mammals such as mice, we will also use the eggs from two other systems: Xenopus frogs and chicken. *Xenopus* is an excellent alternative embryological system. On induction with hormone, each female can lay about 3000 embryos, allowing us to rapidly express different proteins, producing reproducible, statistically significant phenotypic and biochemical readouts. As a comparison, to produce 3000 mouse embryos would require 100 or more adult female mice. Therefore, using one female Xenopus can directly reduce our animal use by 100-fold. Furthermore, harvesting embryos from Xenopus is non-invasive, and the female can be reused after resting; thereby increasing the reductions further.

Similar to Xenopus, chickens are another excellent approach for reducing animal numbers. Because early developmental stages can be acquired in a non-invasive manner, and the embryos are readily accessible, many of our studies of molecular influences on craniofacial development will be first tested in chicken eggs, again, allowing us to reduce the numbers of mice required.

Refinement

Explain the choice of animals 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.

Refinement

Our choice of animal models is designed to refine our experiments and minimise animal use and welfare costs while maximising scientific insights.

We use Xenopus frogs as our first line animal model because Xenopus allows us to test many hypotheses rapidly, while using a very minimal number of animals. Xenopus females lay their eggs externally and can be reused with minimal stress. Because hundreds of synchronous embryos are produced, we can test many scenarios in well-controlled experiments. This approach helps us to rapidly refine our chicken and mouse experiments, and greatly improves the power of our studies. We then move our studies toward chicken embryos, where we can design experiments more focused on the craniofacial skeleton, which in chicken is more analogous to mammals. This allows us to refine our hypotheses prior to moving into mouse, which as a mammal, is our model that is most neurophysiologically similar to humans. Nevertheless, the mouse remains the most powerful system for studying mammalian genetics – our use of parallel model systems allows us to make excellent use of these strengths.

PROJECT 226

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Cell engineered bladder augmentation
Key Words	bladder augmentation, pig immunosuppression
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Some people who have problem with the bladder may require an operation called “bladder reconstruction”. A bladder reconstruction involves either making an entirely new bladder (replacement) or enlarging that person’s existing bladder (augmentation). There are many reasons why someone might need a bladder reconstruction, such as bladder exstrophy, neurological disorders such as spina bifida, posterior urethral valves, accidents or tumours.

In order to perform the operation, the surgeon takes a piece of the bowel or intestine and joins this to the bladder to make it larger.

Although this operation works very well and can provide good urinary control and enhance the quality of life, it is associated with complications, including a long-term risk of cancer.

The aim of the project is to investigate what alternative materials can be used to perform the operation, which may carry a reduced risk of complications. We would like to investigate the possibility of growing a piece of the patient’s own natural tissue in a laboratory. This is what is known as ‘tissue engineering’. This bladder tissue, which has been grown in the laboratory from the patient’s own cells, can then be used for the bladder enlargement surgery.

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)?

Before to be able to apply our technique to children we need to test it in live animals to prove that it works as we expect. Once demonstrated successful we will be able to transfer it to patients (children and adults).

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

We are planning to use a maximum of 42 pigs

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

Animals will undergo surgical operations under general anaesthesia. We are planning to need max 3 – 4 procedures in total (bladder augmentation, removal of bladder conformer, cystoscopy and biopsy, collection and termination). Other possible procedures will include imaging performed at the time of a general anaesthesia. After the surgery animals will be carefully monitored by veterinary anaesthetists and surgeons and will receive full support in case of suspect pain or distress. Animals will receive immunosuppression drugs in order to prevent reaction against human cells and will be daily monitored in order to identify any sign of adverse effect. We are not expecting adverse effects due to the use of human cells.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The aim of this programme is to investigate an innovative technique for bladder reconstruction. We aim to create a new bladder using cells that have been grown in laboratory. The primary outcome is to demonstrate the success of the technique *in vivo,* using human tissues in order to proceed towards realising the first clinical translation and perform this technique in patients who are candidates for bladder augmentation.

Consequently, this work can only be undertaken in live animals under general anaesthesia.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

In related work performed elsewhere[REDACTED], our colleagues have shown that in a normal pig, autologous bladder epithelial (urothelial) cells grown in the laboratory can be transplanted successfully to augment the urinary bladder. In order for us to proceed with this technique to patients, it is imperative that we first show that human epithelial cells grown in the laboratory are able to survive in the environment of the

urinary bladder in vivo, using a) normal human urothelial cells, b) normal human epithelial cells from a surrogate tissue (oral mucosa) and c) human urothelial cells from a diseased bladder. Although we know from the previous work that the surgery is feasible (and was optimised to reduce an initial failure rate due to herniation), the major challenge we face in this project is the need for immunosuppression to allow survival of xenogeneic (human) cells when transplanted into the pig bladder. In order to reduce the use of animals, we propose to 1) use state-of-the-art medication for (human) allogeneic transplantation that 2) has been shown to be non-toxic in pigs (although efficacy is as yet unproven) and 3) to initially test group (a) (normal human urothelium into the augmented pig bladder); only if this proceeds without major complication will we proceed to 4) test (b) and (c).

Refinement

Explain the choice of animals 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.

Refinement

We have undertaken previous work in pigs and are familiar with the surgical technique, which, moreover, is similar to the technique we routinely perform in humans. For that reason, we are familiar with the anatomy and the tissue handling both in the human and the pig. This will ensure we minimise technical errors. The pig model has been chosen due to the similarity in size, physiology and anatomy to human. The method we are going to use is, in principle, the same we currently use in humans and has been revised and optimised with years of experience. Animal suffering will be minimised, as the surgery will be performed under general anaesthesia. During the postoperative period the animals will be carefully monitored both by veterinary anaesthetists and surgeons. A prolonged acclimatisation period of at least 2 weeks will be utilised for the animals (for immunosuppressed animals) in order that they can be trained with treats and tolerate hand-feeding to reduce stress.

Following recovery the animals will be regularly assessed for signs of pain, dehydration and complications of the surgery with an experienced animal care staff (NACWO) being available on the premises or on call and a researcher being on call.

PROJECT 227

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Mechanisms of Acute and Chronic Inflammation
Key Words	Inflammation, Lung Diseases
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Inflammation is a normal biological process that the body uses to protect itself against diseases and for restoring damaged tissues back to normal function. However, when inflammatory processes are poorly controlled or directed against normal bodily functions they are harmful to the affected individual and result in diseases such as Asthma and Chronic obstructive pulmonary disease. The inflammatory response involves directing specific white blood cells to sites of disease where they interact with other cells to secret substances which allow the cells to divide and fight the disease. However, how the cells are directed to and function at sites of disease and damage are not well understood. The overall objective of our investigations is to identify molecules that start and propagate the inflammatory response.

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)?

Understanding the mechanisms involved in the inflammatory response is fundamental to analysing the processes of infectious disease control on the one hand and inflammatory diseases on the other. This understanding is critical for the design of new drugs for common inflammatory diseases for which there are currently few effective treatments.

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

We expect to use only mice (approximately 30000) over 5 years, amongst 20 researchers.

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

The following procedures are expected to cause some moderated discomfort, that animals will fully recover from; bone marrow ablation and reconstitution (tiredness and reduced appetite), splenectomy (pain) and pathogen exposure (weight loss). Other procedures, such as administration of substances, assessment of lung function and taking of blood samples cause mild transient discomfort and no lasting harm. We do not anticipate any severe adverse events. However, we will monitor animals for recognised physical and behavioural changes that indicate ill health. Mice displaying two or more of these will be humanely killed. All experimental mice will be humanely killed at the end of the experimental procedure.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Due to the multi-cellular interactions involved in the inflammatory response and in inflammatory diseases such as asthma the responses cannot be adequately or fully mimicked by in vitro studies alone. It is critical to perform these studies in mammals since there are significant differences between the biological systems of frogs and fish to that of humans. Where possible we will complement the in vivo work with experiments using in vitro culture systems taking advantage of isolated human cells and cell lines to investigate selected pathways identified in the in vivo models. Throughout the project, where possible, non-animal experiments will be employed. This will include developing in vitro systems such as ‘lung on a chip’ and exploiting in silico technologies and databases where appropriate.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We have developed our experiments so that we can measure multiple parameters in each animal, thus maximising the information gained from each experimental group and minimising the number of animals used. Combining tests in the same mice allows the data gained to be correlated directly, rather than inferred. Based on previous experience we have calculated the minimum number of animals needed to see desired effects using robust statistical analysis. Also, all experiments will be conducted in accordance with the NC3Rs' ARRIVE guidelines. All of which help to minimise variation and avoid unnecessary repeats.

Refinement

Explain the choice of animals 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.

Refinement

We have chosen the smallest animal possible to represent human disease. While the models chosen closely represent the features of the human disease in the treated animals, they are the least severe and do not promote undue distress to the mice. We constantly monitor animals for signs of ill health and work closely with animal care staff and veterinary surgeons to ensure the best possible husbandry and welfare for mice under procedure. Analgesia is routinely provided to all animals when required.

PROJECT 228

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Neuronal cell development and survival
Key Words	Motor neurone disease, Axonal transport, RNA metabolism, Cell biology
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Our objectives are: 1) To understand how defects in the components of the intraneuronal transport and signalling systems lead to the death of motor neurons in motor neuron diseases. 2) To investigate the underlying mechanisms of the roles of proteins implicated in motor neuron disease in response to DNA damage and to elucidate how defects in these proteins could affect the expression of other genes.

Our study will contribute towards our understanding of the mechanisms of motor neuron death caused by defective intraneuronal transport or response to DNA damage. Therefore, our findings will benefit the scientific community with a broad range of interests in neurological conditions. Moreover, working from the mouse models of motor neuron disease to mouse primary cells and neurones derived from reprogrammed mouse skin cells, will aid the understanding of the mechanisms of disease onset and progression. Using this knowledge in human derived fibroblasts and neurones and applying this information back to human conditions and for cross species comparisons at the cellular and neuronal tissue levels will set a paradigm for the effective use of both the mouse and human-derived cells as valuable model systems.

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

This research will benefit patients and their families, who have been affected by motor neurone disease, hereditary motor neuropathies, and some cases of intellectual disability; and health professionals, who work with the above mentioned patient groups. The benefit from the outcomes of this study could be immediate, as

our findings could inform the beneficiaries about the causes and basic mechanisms of the disease. In the longer term, this project will contribute to: 1) our understanding of the relationships between defective axonal transport or DNA repair response with abnormal neuronal cell function and development; 2) and hence, discovering novel drugs and more effective treatment of the above mentioned diseases and perhaps other related disorders; 3) validating the promising drug targets in preliminary pre-clinical settings; 4) informing patients and ensuring best possible care planning.

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

Mice,6000 over five 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?

A proportion of the animals used in this study are transgenic mice that start showing signs of a progressive muscle weakness in their limbs. The level of severity of the phenotype in these mice is substantial, as this is a progressive condition which leads to paralysis and it is crucial for this research to obtain tissues from all stages of the disease in order to pinpoint the correct pathway that is impacted, or the efficacy of the drug. To minimise the animal suffering we monitor these mice twice a week between from the pre-symptomatic stage. Mice with signs of paralysis will be given dry mash and gel blocks and their food and water intake will be monitored daily. Mice will be weighed once and checked twice every day till end point (righting reflex within 30s is not observed; or 15% loss of body weight) is established. End-stage mice will be monitored 9am-5pm. If the mouse shows sever symptoms, then it will not be kept and will be culled humanely as specified by the Home Office. No mice with severe symptoms will be kept overnight. Another group of mice showing adverse effects in this study exhibit an abnormal gait but have normal feeding behaviour and life span.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Motor neuron disease targets neurons in the brain and spinal cord and thus it is impossible to have access to these tissues during the development of the disease before the post-mortem stage. This would provide us with data about the very late stages of the disease. Although we will be using skin fibroblasts isolated from patients and reprogrammed cells, we will still need mouse models to have access to tissues at all stages of life and for culturing primary neurons.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will maintain and breed just enough animals for providing us with required tissues and cells for generation of data which are statistically sound.

Refinement

Explain the choice of animals 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.

Refinement

Sequencing of the mouse genome has revealed that ~99% of mouse genes have a homologue in the human genome and that for ~80% of mouse genes an analogous (orthologues) gene exists in the human genome. In addition, human and mouse have common biochemical pathways.

Because of the above properties several large international mutagenesis programmes have been generating mutant mice that could serve as model systems for late onset human disorders such as motor neuron disease.

The mouse clearly does not have the same physiology as humans, but does, largely, share the same biochemical pathways as well as genes. Thus we can work with mutant mouse models of human motor neuron degeneration to highlight and interrogate the proteins and pathways that are involved in motor neuron disease.

To minimise the animal suffering we monitor the animals which show signs of muscle weakness or paralysis twice a week between 100 – 120 days of age. Mice with signs of paralysis will be given dry mash and gel blocks and their food and water intake will be monitored daily. Mice will be weighed once and checked twice every day till end point (righting reflex within 30s is not observed; or 15% loss of body weight) is established. End-stage mice will be monitored 9am-5pm. If the mouse shows sever symptoms then it will not be kept and will be culled humanely as specified by the Home Office. No mice with severe symptoms will be kept overnight.

PROJECT 229

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Animal models of inflammatory bowel disease
Key Words	Inflammatory bowel disease
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall objective of this project licence is to identify compounds and antibodies that could become new medicines for the treatment of inflammatory bowel disease (IBD). To achieve this objective potential new medicines will be tested in rodents in which disease has been generated which has similarities to human disease, whilst keeping any distress and discomfort experienced by the animals to a minimum.

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)?

Inflammatory bowel disease affects the intestines such that they become inflamed. Abdominal pain, diarrhoea, weight loss and bleeding from the intestines are typical symptoms. Some medicines can reduce inflammation to some extent, but they are not effective in all patients and have many side effects. New medicines are therefore needed that effectively treat more patients with this common severe disease with lower toxicity than currently available treatments.

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

This project will use mice and rats. It is expected that less than 500 animals will be used each year for the 5 year duration of this project.

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

The animals will undergo procedures that may involve injections and they may experience moderate discomfort as they will experience some symptoms of disease, such as weight loss and diarrhoea. At the end the animals will be killed in a humane way by trained staff.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

A significant proportion of drug discovery work is carried out using cells and cell lines (in vitro), with many thousands of potential drugs being screened to identify the most promising compounds. However, IBD is a complex disease that involves many different tissues and cell types and therefore further testing in animals is required and there is no alternative but to use animals in which disease symptoms have been induced.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

To ensure the fewest number of animals are used, only the most effective drugs that have been pre-screened for activity in vitro will be examined in animals.

Refinement

Explain the choice of animals 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.

Refinement

Only rodents (mice and rats) will be used in this project, as these are the lowest animals on the evolutionary scale in which disease similar to human disease has been generated. Anaesthesia will be used where appropriate and welfare score sheets are used to monitor disease to ensure any discomfort is not severe. There are also limits to the number and frequency of any injections and blood sampling. All procedures have been ethically reviewed and all animals undergoing procedures will be well looked after by trained staff that work closely with a veterinary surgeon.

PROJECT 230

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	In vivo imaging of cancer disease models
Key Words	Imaging, Cancer, Diagnosis, Therapy
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

In the UK, more than 330, 000 patients are diagnosed with cancer each year. This forecast is set to rise to more than 425,000 annually by 2030. Further improvement in the management of cancer patients requires **early detection** of small tumours and metastases (cancer spread), treatment selection informed by individual patient and tumour type (**outcome prediction**), and **monitoring of treatment outcome** in the individual patient to make decisions about changes to method or purpose of treatment. Ideally, this diagnostic information should be gathered as non-invasively and cost-effectively as possible. Imaging techniques can non-invasively detect disease and measure response to treatment.

This project aims to develop non-invasive imaging methods and agents to improve diagnosis and treatment of cancer. The programme spans “bench to bedside”, covering a variety of novel imaging agents and methods, including combinations of imaging technologies.

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 project outlined will allow us, with the help of disease models, to develop novel imaging techniques and devices that will help to better diagnose, understand and quantify the severity of cancer development and spread and also improve current interventional procedures for the treatment of cancer. It thereby would allow us to more efficiently treat patients with cancer and help to assess how patients respond to treatment (thus avoiding debilitating and expensive treatments in patients who will not benefit). This knowledge will help us in identifying patients that are at high risk of cancer spread and provide the appropriate treatment to patients based on more quantitative and objective measures. Ultimately this work aims to improve cancer treatment by early detection and improve monitoring of cancer spread over time.

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

Mice (7,000) and Rats (1,750) will be used 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 levels of severity? What will happen to the animals at the end?

Generally, no adverse events are expected to be caused by the imaging methods used. Any adverse events expected are related to induction and maintenance of anaesthesia (animals may die from respiratory depression <1 -2 % and/or hypothermia). Mild discomfort may ensue from injection of substances (however, where possible, this is generally done under anaesthesia) or withholding of food prior to imaging (as done in the clinic) but efforts are being made to optimise anaesthesia, administration of substances and avoid unexpected adverse effects &/or deaths. Adverse events may occur related to surgery designed to: implant or remove tumour tissue or implant pellets (for slow controlled delivery of substances) but these will be minimised with good aseptic surgical techniques, good monitoring measures, and painkillers. Symptoms of tumour development and progression which develop over time in genetically altered animals or due to disease induction by tumour cell implantation will occur but again careful monitoring will minimise as much as possible, any pain and suffering and distress. All animals will be humanely killed at the end of the experiments and tissues taken for further analysis. However, if at any point during the studies the animals reach a predetermined end-point at which pain, suffering and distress can be avoided or minimised, then these animals will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animals have to be used because 1. Data generated from this body of work may be used to inform whether to go forward to human clinical trials/studies 2. To validate mode of action of novel compounds, experiments are required which cannot be conducted in humans for ethical and scientific reasons. 3. Bio-distribution in whole organisms (i.e. tracking the injected agents route/ accumulation and excretion through the body), with intact biological barriers and excretion mechanisms, is key to clinical use. Non-animal alternatives cannot replace the complexities of the interactions of these probes in whole body systems or with realistic models of cancer.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Preliminary screening (i.e. cells or tissues used in a controlled environment outside a living organism) will eliminate unsuitable candidates at an early stage and thus these substances will not progress to animal studies, thus reducing the numbers of animals used in this project.

Imaging allows repeated observations/measurements over a period of time (longitudinal study) on the same animal, with humane killing only at the last time-point. The use of imaging to determine bio-distribution of novel contrast agents in the life of the same animal rather than conventional killing at sequential time points, with removal of tissues for analysis is a major contributor to reduction of numbers. Thus, if a longitudinal study involves six time-points, the numbers of animals are reduced to one sixth by use of repeated imaging. Since each animal serves as its own control to compare different time-points, the data obtained are statistically more robust (reduction), requiring fewer animals. Moreover, distribution of contrast agent within organs, not just between organs, is obtained, and unexpected uptakes that may not be detected by conventional methods can be found by whole body scanning. All these attributes of imaging contribute to a greatly improved benefit:cost ratio (benefit = data quality and quantity, cost = animal numbers, procedures and their severity).

Refinement

Explain the choice of animals 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.

Refinement

Species: Mice and rats are the species of lowest neurophysiological sensitivity that provide the necessary size compatible with the scale of resolution or movement associated with the techniques being studied. Resolution of the whole body imaging techniques is of the order of 0.5 - 1 mm. Distribution within smaller animals will be beyond these limits.

Pilot studies are small experimental groups which help us to decide quickly how best to design a statistically and scientifically valid experiment. Thereby helping develop better larger study design and reduce possible suffering. Generally, inhalation anaesthesia will be used to minimise any transient pain or distress and where possible, used for blood sampling, contrast injection, weighing and combined with imaging techniques where it is mainly used for restraint. In addition, there would be full and complete recovery between periods of anaesthesia and/or food withdrawal; rehydrating of animals during long imaging sessions; monitoring of respiration and/or cardiac function and maintaining body temperature during imaging. These

steps/measures will optimise the animal's welfare whilst undergoing these procedures. By the very nature of the work involved in this project animals will develop cancer. Therefore careful monitoring will occur and pain relief will be administered as required and under veterinary direction. Animals will be humanely killed at the end of the experiment or before then, if the humane endpoint is reached and tissues used for further examination.

PROJECT 231

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Electrical Impedance Tomography of nervous system function
Key Words	Electrical Impedance Tomography, Neuroimaging, Electroceuticals, Epilepsy
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
Yes	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Electrical Impedance Tomography is a recently developed imaging technique, with which images of the internal impedance of the subject can be rapidly collected with rings of external ECG-type electrodes. It is fast, inexpensive, portable and sensitive to physiological changes which affect electrical impedance properties. Set against this is a relatively poor spatial resolution so that small technical errors in recording translate into large errors in images. For about two decades, satisfactory images have been obtained of changes over time related to emptying of the stomach and breathing of heart output in the chest. The main work of our group has been to adapt existing EIT designs for the demanding application of imaging changes in the brain due to conditions like stroke, epilepsy or normal physiological brain activity. For the former, its portability and low cost would give it unique practical advantages over existing methods; it also has the potential for providing images of fast electrical activity in the brain over thousandths of a second which would constitute a revolutionary advance in neuroscience technology. Engineering challenges are that the skull acts as an insulator so that recoded signals can be low and imaging in acute stroke is demanding. Approaches used to overcome these problems employ advanced engineering and mathematical methods. We conduct as much research as possible using computer modelling or saline filled tank studies. However, the demanding conditions encountered in human studies can only be approximated with in vivo animal studies. The great majority are undertaken in terminal studies in the anesthetised rat to allow optimisation of technical parameters before application in human studies.

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)?

Overall, innovations in hardware and image reconstruction algorithms developed in our group enable accurate images in tanks and in experimental animals with electrodes on the brain or in nerves; the next challenge is to see if recent technical improvements allow us to collect clinically useful images in human subjects with epilepsy or diseases that could be treated with implanted miniature devices that stimulate autonomic nerves. What types and approximate numbers of animals do you expect to use and over what period of time? We anticipate needing 300 terminal studies over the 5 years of the project licence. A small number of up to 50 may be undertaken in recovery studies where electrodes have been inserted to record from the brain or nerves.

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

We anticipate needing 300 terminal studies over the 5 years of the project licence. A small number of up to 50 may be undertaken in recovery studies where electrodes have been inserted to record from the brain or nerves.

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 studies are in terminal experiments. For terminal studies, animals are induced by painless standard methods, usually just by painless inhalation of anaesthetic vapour and thereafter do not experience any pain as they do not recover. For recovery studies, there will be post-operative analgesia and antibiotic administration with careful monitoring of animal health and behaviour to ensure these are effective. The surgical procedure is designed to minimise suffering and will closely resemble the method used in human subjects who undergo electrode implantation for presurgical evaluation for epilepsy surgery. Animals will be closely monitored and standard protocols for analgesia and infection controls will be applied. Further physiological studies – visual or sensory stimulation – are not painful and are standard methods used widely in clinical medicine which are well tolerated by human subjects including children.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The method has small signals which are at the limit of detectability. Previous experience has shown that optimisation of technical parameters is only possible under laboratory conditions in experimental animals where prolonged recording and

adjustment of variables is possible. As soon as this can be accomplished, we plan to move to human studies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

In all experiments, we undertake constant review to ensure that only the animals needed to achieve technical goals of satisfactory signal and image quality are used. All projects are discussed with senior statistical advisers to decide on the minimum number needed to provide statistical significance of the accuracy of the images. The data produced comprise EIT imaging data sets which are compared to independent gold standards such as MRI or CT for the brain or measurement of nerve or brain activity with electrodes. The data are usually analysed objectively using a standard statistical method for medical images, Statistical Parametric Mapping.

Refinement

Explain the choice of animals 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.

Refinement

Rats have been selected because they have been extensively investigated for such changes in the literature. Their brains are of a reasonable size for surgery but they are still small laboratory animals and so relatively easy to manage.

PROJECT 232

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Targeted Tumour Therapy: To assess the ability of anticancer agents, either alone or in combination with other anticancer agents, to treat or prevent tumour growth.
Key Words	Tumour, Targeted Therapy, Immunotherapy
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production

	conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

1) To assess the growth of different tumour types (for example melanoma, bladder cancer, ovarian cancer, prostate and breast cancer) within a mouse/rat model.

2) To assess the use of anti-cancer agents, alone or in combination to kill tumour cells.

3) To investigate the mechanisms and cell death pathways induced by anticancer agents, and their ability to prime the immune system against the tumour 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)?

Current cancer therapies target all the cells in the body including normal healthy cells. By creating novel agents that target only tumour cells this should reduce unwanted side effects experienced by patients. • To develop and investigate the anti-tumour potential of new agents, as well as new unique combinations to provide more effective cancer therapies. • Use Oncolytic viruses to create a cancer vaccine which will target the primary tumours and train the immune system to recognise tumour metastases which have spread to other parts of the body. • The findings of this research will be made available to other scientists and clinicians via Publications and presentations at national and international meetings thus advancing the knowledge of this area of research.

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

This project uses laboratory rats and mice and we plan to use up to 4925 animals over the five year period.

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

The majority of this work will be carried out in models in which the tumours are seeded onto the flank of the animal (protocol 1-4). The flank models will cause minimal suffering to the animals. We will also be conducting a few experiments in which tumour cells are injected into the vein of the animals (protocol 1-4) or directly into the bladder (protocol 5). All models will be rigorously monitor according to each protocol's clinical scoring system. Based on our actual severity assessment data from last year 2015, 95.38% of animals shown mild severity. All mice will be euthanased at the end of the experiments (based on defined experimental or humane endpoints).

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

One of the key aspects to our research is to study the role that our novel anti-cancer agents play in immunogenic cell death and modulating the immune system. For this we require a live animal with a fully functioning immune system.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used in this study has been estimated based on what would be required to obtain statistically valid results. This was calculated after reviewing similar published data and seeking the advice of experts in statistics and experimental design. Following the initial experiments, estimates for the number of animals per group will be reviewed and adjusted as necessary.

Refinement

Explain the choice of animals 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.

Refinement

Mouse cancer models represent a mammalian system that is well characterized and available in defined genetic backgrounds, greatly reducing the number of variables that could influence experimental outcomes. There is strong evidence in the literature which, is backed up by our own experience, shows that tumour flank model and tumour model infusing cancer cells into the bloodstream or the bladder, cause minimal distress to animals during implantation and growth of the tumours. In all tumour models we monitor changes in: respiration depth and pattern (laboured breathing), hunching, an altered demeanour, tumour dimensions and tumour ulceration. To minimise suffering of the animals timely monitoring and careful observation of the mice will ensure that any harms are kept to a minimum and when they occur, dealt with promptly.

PROJECT 233

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Neuronal communication in the brain of mice
Key Words	Brain, cortex, synapse, neuron, tau
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

A central goal of neuroscience research is to understand how the brain processes external and internal stimuli to coordinate cognitive and behavioural processes. Ultimately, this understanding must relate behaviour to the activity patterns of neurons and their synaptic connections within key circuits of the brain. Elucidating how the activity of these circuits becomes abnormal is also crucial to understanding pathological situations such as neurodegeneration. This project deals with these quetions in an unusually direct way - by observing the activity of neurons and synapses in the brains of live mice as they process sensory stimuli (1-4). Optical methods such as multiphoton microscopy now provide the resolution required to image neuronal and synaptic activity in awake animals and we will use these methods to make a circuit-level analysis of sensory processing and neuropathologies such as Alzheimer’s Disease.

Our aim is to understand how nerve cells and their synaptic connections convey information (e.g visual or spatial) during health and disease.

Our key questions are:

1. What is the nature of the neural signals by which information is processed and transmitted in the visual system and hippocampus in awake behaving mice?

2. How is the transmission of neural signals at the synapse altered by changes in behavioral state of the animal?

3. How is the processing and transmission of neural signals altered in neurodegenerative disease states

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 have benefits in three broad areas: 1) By elucidating how different types of neuron respond to different types of visual stimuli, it will yield a greater understanding of how the brain executes vision. 2) By concentrating on imaging the activity of synaptic connections, it will reveal how these key neuronal compartments alter visual signals and how they alter the transfer of neuronal signals when the brain enters different states, such as switiching from "sleep' to "alert". 3) By studying how the operation of neurons and synapses is altered by the accumulation of proteins that are known to cause neurodegeneration, we hope to suggest novel therapeutic targets and strategies.

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

This project will use mice. Approximately 3000 will be used over the course of the project.

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

The large majority of the genetically-altered mice used in this project usually show no adverse effects, particularly at the ages used in our experiments. However, about 5% of mice will be mutants that develop protein depositions in the brain leading to neurodegenerative changes of moderate severity. Some animals undergo surgery to allow us to implant a window through which we can image their brains. They may require post-operative pain-killers but are usually fully-recovered and alert a few minutes after the procedure. Occasionally, this surgery may also include the implantation of an optical fibre or cannula. After recovery from surgery, animals are gradually habituated to the experimental equipment, on which they have their head fixed in place but can run on a ball. They will be rewarded, for example with sucrose, and stress will be minimised by accustoming them gradually to the apparatus, but nevertheless, this can sometimes be somewhat stressful for the mice. At the end of all experiments animals will be humanely killed and where applicable tissues collected and analysed. If animals are suffering for any reason before the end of the experiment and do not respond to treatment, they will also be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The research proposed here requires the use of animals and animal tissue. The mechanisms underlying the transmission of signals in the brain are complex and involve interactions between different cell types. Studies in cell culture are

uninformative as to the physiological properties of these processes, as the properties of the various neurons and their interactions are altered by the tissue culture process. Nevertheless, we will use a number of different experimental preparations, minimising the use of living animals where possible. Where experimentally relevant, studies will use *ex vivo* brain slice preparations to study how sugnals are transmitted across synapses.

But to investigate how synapses in the brain contribute to the processing of information (e.g visual information in the visual cortex or spatial information in the hippocampus), we will need to work *in vivo*. This is the essence of our approach: to use the actual, unperturbed, neural circuit as far as possible. Cultures of neurons cannot see or navigate in space or carry out behavioural tasks that reflect the normal functions of the brain, and are therefore not an adequate substitute to understand how the retina or brain works.

As the function of neural circuits is profoundly altered by anaesthesia, many experiments will study unanaesthetised animals. This is also required to study processes such as motivation states. We will always seek to minimise animal use, however, and to maximise the information gleaned from every animal used

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will collect multiple data from each animal, thus minimising the number of animals required. For example, in vivo imaging data of neuronal and synaptic activity will be collected from many hundreds of neurons in the one animal, from several regions of the cortex or hippocampus. The numbers of animals to be tested will be the minimum number required to obtain statistically reliable results, based on previous experience in the laboratory, and from published findings.

To preserve important mouse genetic lines without having to hold stocks of live animals for extended periods we will instead freeze embryos that can later be implanted into a female mouse. The surgical procedures for implantation are demanding and require practise so on occasion it may be necessary to train with re-implantation of un-manipulated oocytes, embryos or blastocysts.

We are also using carefully designed studies that are statistically sound to minimise the number of animals used.

Refinement

Explain the choice of animals 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.

Refinement

Mice have similar brain structures and functions with humans and can be bred to express proteins that allow us to see different cell types and the amount of brain activity. They are also small enough to be able to image their brains under a microscope.

The experimental and disease models have been designed to provide robust data while minimising animal suffering. Firstly, they use surgeries from which the animals rapidly recover. Secondly, they study the onset of disease processes. This allows the key triggers to be identified before multiple other processes go wrong, and also means that at these early stages, animal well-being is barely affected.

Animal welfare is monitored throughout the experiments, and animals are humanely killed where necessary.

Importantly for this project, a number of transgenic mouse lines are available, which will allow visualisation of specific types of neuron in the brain and the measurement of fluorescent signals when neurons are activated . Mice have been well-studied to investigate brain function and the sense of vision, as well as diseases such as Alzheimer's Disease, so that these results will be readily integratable within the field and should prove more translatable to humans than studies using lower order vertebrates. Finally, the small size of mice means that more of the brain can be observed using current microscopes than is possible in larger species.

Using a chronic cranial window for imaging involves surgery from which animals quickly recover, and then are stable for several months with usually no decrease in life expectancy or quality of life due to the surgery. Animals will be gradually accustomed to the imaging apparatus and will be rewarded (e.g. with sucrose solution) while under the microscope to decrease any aversion and stress associated with the apparatus. The transgenic mouse models to be used either have no adverse phenotype or, as in mouse models of neurodegeneration, they present some of the symptoms seen in human conditions such as Alzheimer's Disease. The use of such mouse models of human diseases is making possible the scientific studies from which cures will be found.

Where surgeries are carried out, peri- and post-operative analgesia will be used to minimise pain. Animals will be housed with appropriate environmental enrichment, and post-operatively this will be adapted to ensure that there is no chance of catching the cranial implants, while maintaining an interesting environment.

PROJECT 234

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Circadian mechanisms underlying organ rejection
Key Words	Circadian, Immunity, Allograft, Transplant
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Rejection of a transplanted organ remains one of the biggest challenges in transplantation. Despite use of drugs to supress the immune system the transplanted kidney will be rejected in 10% of kidney recipients in the first 12 months, damaging the organ. This programme of work will identify whether organ rejection is under local control from our body clock. It will then go on to identify which pathways and cell types are involved.

The twenty-four hour circadian clock

Virtually all aspects of how our body functions are mapped onto 24 hour rhythms, orchestrated by internal body clocks. These internal clocks help anticipate changes in our environment so that our bodies can respond appropriately. Circadian dysfunction is now considered to be a contributory factor to the incidence and severity of a wide range of body functions including sleep, blood pressure, heart rate and immune function.

The circadian clock and immune function

There are clear links between circadian rhythms and the function of our immune system. Firstly, immune cells and immune organs possess a clock which ticks over. Furthermore, immune cells demonstrate variation in their function according to time of day. This is exemplified in animal experiments e.g. the outcome of pneumonia is altered by what time of day the illness is contracted.

Organ Transplantation

Organ transplantation is performed due to end stage organ failure. The main complications after organ transplantation are due to the immune system attacking the transplanted organ termed “rejection”. Currently most transplant recipients have

to take immunosuppressant drugs to prevent or treat this rejection. These drugs all have significant side effects causing a reduced quality of life for the transplant recipient. Despite this, the drugs have to be used since current demand for organs outstrips supply reducing the chance of re-transplantation.

Objectives of the project:

This project is investigating whether timing of transplantation affects rejection of the organ. In addition we will study how transplantation itself can affect the body clock and the downstream consequences of this disruption on the immune system.

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 benefit of this project relates to new knowledge about circadian mechanisms altering rejection of the transplanted organ. In particular, the work outlined in this project will establish whether the circadian clock affects transplant organ rejection and provide detailed information about which components of the immune system (i.e. which cytokines/chemokines and which inflammatory cell types) are impacted by the circadian clock after transplantation. The aim is to publish the findings in academic journals, since the information will be of interest to both scientists and clinicians. The more long term and substantial benefits of this research relate to the possibility that new molecular targets may be identified, for which pharmaceutical products could be developed. Finally, a greater understanding of how the clock impacts on organ rejection after transplantation may change clinical practice since it is now possible to preserve organs outside of the body allowing transplantation to be performed at the optimal circadian time.

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

2600 mice over 5 years

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

Breeding: This project covers the breeding of a number of genetically altered lines of mice, which have alterations in their circadian clock. This procedure is mild, as these lines of mice are unlikely to show any adverse effects in their normal state. Allograft model: Under recovery anaesthesia, mice will receive a skin graft from a donor mouse either on the hindlimb, abdomen or flank. Depending on the genetic background of the donor versus recipient, this may result in graft rejection. Great rejection may result in localised cell death and tissue damage at the graft site. Animals will be checked regularly for signs of graft rejection and for any signs of adverse effects. This is a moderate procedure, and mice will be given pain relief post surgery and group housed whenever possible to minimise stress. Surgical intervention: Animals may be surgically treated (under recovery anaesthesia) by

implanting a small pellet under the skin that slowly releases a hormone. Additionally, the adrenal glands may be removed (under recovery anaesthesia) in order to abolish the rhythmic release of the anti-inflammatory hormone corticosterone into the blood. Corticosterone may be replaced by timed administration to either invert the rhythms or maintain levels at the peak or nadir. All these procedures are moderate and well tolerated and not associated with any adverse effects. Pain relief will be provided during and after surgery. At the end of the experimental protocol, animals will be culled using an appropriate and human method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Currently there is no *in vitro* or *in silico* model of rejection, as this process involves a number of cell types and recognition of foreign tissue. Whilst some of our work will be done *ex vivo* and *in vitro* (where we will try and recreate a specific part of the rejection process that we are seeing in the animals, using cells, tissues and cell lines), it will also be necessary to use *in viv*o models. Mice will be used for these studies as they possess the complex immune and circadian system (similar to humans) needed to address our aims. Models of allograft rejection have already been established in this species. Furthermore, transgenic mice are available to us which lack the circadian clock in specific immune cells and these animals will greatly facilitate this work.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Animal number:

Initially pilot studies will be carried out see how many animals are needed to generate robust data. After this information has been collected we will work with our biostatistician in order to ensure that the experiments use the least number of animals to produce robust results.

Sample analysis:

Animal numbers will be minimised by utilising technologies which extract the maximum amount of information from single samples. This vastly broadens the array of data that can be collected from a single experiment and removes the need to repeat experiments.

Tissue archiving:

Tissue samples from *in vivo* experiments are routinely archived, which allows us to build a bank of samples which can be accessed by others in the wider grouping, and avoids experiments unnecessarily, thus reducing animal usage.

Refinement

Explain the choice of animals 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.

Refinement

Choice of animal and model:

Mice will be used for these studies as they possess the complex immune and circadian system (similar to humans) needed to address our aims. Models of allograft rejection have already been established in this species and have been used in the past to assess the efficiency of immunosuppressive agents, and to investigate the biological effects of blocking antibodies.

Minimising welfare costs:

Where surgical procedures are to be carried out, analgesia will be provided during and after surgery. Animals will be group housed wherever possible to minimise stress, and provided with environmental enrichment. When an animal is undergoing a procedure, they will be monitored regularly for skin graft rejection (where appropriate) and for any signs of adverse effects.

PROJECT 235

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Breeding, Maintenance and Validation of Genetically Altered Rodents
Key Words	Genetically Altered Animal, Rodent, Transgenic
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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Our primary mission is the discovery, development and provision of new therapies for the treatment, prevention or cure of diseases and disorders in humans. The studies enabled by this program of work are of vital importance to help inform us of the development and safety of potential treatments, therapies and cures prior to their progression through the drug discovery process. Genetically Altered animals, particularly rodents, are currently in widespread use in biological and medical science and have been shown to be of great value in understanding the function of genes and pathways in a wide variety of biological, physiological and pathological processes. The use of Genetically Altered animals allows for specific gene traits to be studied in a complex physiological environment, where there are currently no alternative lab-based approaches that are appropriate to replace these animal studies.

The aim of this project is to maintain, breed and genetically validate colonies of Genetically Altered strains of mice and rats, requested for and supplied to other project licences, scientifically and ethically justified to further research programmes in areas such as neuroscience, cardiovascular, inflammation, respiratory and oncology. By restricting breeding and maintenance of Genetically Altered animals to this project, we can ensure they are maintained by an experienced team of licensed technicians and named animal care and welfare teams with the objective to provide a high standard of animals with a known genetic and health status, whilst closely monitoring and limiting the generation of animals through strategic and robust breeding processes.

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)?

Genetically Altered rodents have made significant contributions to human biological and medical research, e.g. Neuroscience, cardiovascular, immunological, respiratory and oncology research and led to supporting the development and testing of novel therapies, treatments and cures for diseases and disorders affecting humans. The ability to import, breed and maintain Genetically Altered animals under a single project will allow us to use the vast experience of our technical staff that have worked in this area of science for many years and networked with a broad group of colleagues in the scientific field to ensure that the highest standard of supply and monitoring of animals prior to them being used for research based studies. Maintaining breeding colonies within a single centralised facility allows the close monitoring of genetic drift, breeding performance, deviation from normal behaviour and health status to ensure that the Genetically Altered animals that we supply are appropriate for the further scientific use that they were ethically and scientifically requested for. A centralised project covering breeding and maintenance of Genetically Altered animals ensures that a minimum number are used by avoiding unnecessary duplication of breeding colonies along with stud and sterile males keeping the use of animals to a minimum.

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

We expect that up to 39,000 mice and 12,000 rats will be used over 5 year licence duration

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?

[REDACTED] the majority of animals used in this project will involve the breeding of Genetically Altered rodents, predominately mice that will exhibit no observable difference beyond that of the genetic background they were generated on. This will involve the natural pairing and rearing of rodents coupled with the need to take small tissue samples to allow their genotypes to be established. The effects of the genetic alterations for the majority of animals will be negligible and of mild severity at most. A small number of Genetically Altered rodents (expected to be less than 5%) may have abnormalities causing moderate severity. Following advice from named veterinary surgeons and experienced staff, these effects can be minimised by appropriate breeding, husbandry and/or veterinary measures and for each instance measures to minimise the effects will be ethically reviewed and documented prior to that procedure being progressed. The licence also allows for the generation, collection and subsequent implantation of pre implantation stage embryos into recipient mice, this will involve a small surgical procedure. Surgical interventions are performed under general anaesthesia with appropriate pain relief to minimise the effects of surgical procedures. Administration of substances and collection of blood or other

tissues and mating procedures will follow published good practice guidelines and should not result in more than any transient temporary discomfort, suffering or distress to the animals. Animals will be transferred to alternative projects where Home Office permission has been granted for their use. Those that are not transferred may be maintained and bred under the authority of this project licence and will be humanely killed by a Schedule1 method.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The use of Genetically Altered rodents allows for specific gene traits and disease models to be studied in a complex physiological environment. This cannot always be adequately achieved by non mammalian species (e.g. fish, drosophila, nematodes) or by the use of non animal methods due to complex physiological interactions within a living system or being translatable to the human condition or adequately predict the human response to treatments.

The use of each Genetically Altered rodent model will be scientifically and ethically reviewed through a robust process with input from a broad group of scientific and named animal care and welfare staff , and during this process the assessment of alternative species or non animal approaches will be debated to ensure these have been considered for their application - Genetically Altered rodents will only be progressed if the scientific goals cannot be met by alternative methods.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Breeding and maintenance of Genetically Altered animals within this single licence will be optimised using the combined knowledge and experience of a team of technicians and animal care and welfare staff to reduce the number of animals needed to provide the genotypes required.

To minimise maintaining Genetically Altered strains beyond their current use, we will use cryopreservation techniques at the earliest time possible. Sperm freezing wherever possible will be used instead of embryo freezing as this will reduce the number of animals required to cryopreserve a Genetically Altered strain.

All steps in every process will be carefully monitored to minimise numbers of animals generated. Unnecessary creation/importation of Genetically Altered animals will be

avoided by database and literature searches to ensure the required strain is not already available for our use. The background strain used will be appropriate to the research. A centralised service is administratively efficient, with breeding controlled to produce batches of animals as needed and any excess made available for use for different scientific projects.

Careful consideration and advice from experienced staff and veterinary surgeons for importation of animals into our facility and maintenance of high health status barrier, will allow access to animals of known health status minimising experimental variation caused by disease leading to more consistent and reproducible results.

Refinement

Explain the choice of animals 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.

Refinement

The species used on this licence will be predominantly mice but as the use of novel genome editing techniques become established rats will also be used if they are justified to be more appropriate for the scientific research programme. The use of animal models, species and strain will be justified prior to any animal use, following a robust scientific and ethical review process justifying why the animal model is required and why alternatives cannot be used to meet the goals of the scientific programme of work which will be authorised under the authority of a separate project licence.

The animals will be cared for by dedicated animal technologists and animal care and welfare staff who have the expertise and skills required in the breeding of the animals and are able to asses any welfare problems and abnormal behaviour that may occur at an early stage and determine appropriate end points in consultation with the Named Animal Care and Welfare Officer (NACWO) and Named Veterinary Surgeon (NVS).

To ensure high levels of care and welfare of the animals we have trained and competent [REDACTED] experienced in a wide range of techniques. Surgical procedures will only be performed using appropriate aseptic techniques, analgesics and anaesthetics to minimise discomfort. Through review of literature and by continual personal development, refined technical procedures will be reviewed and used to minimise the impact on the individual animal.

Animals will be routinely group housed with littermates or in compatible age/sex groups. Where group housing poses welfare concerns to the animal (e.g. through fighting of incompatible cage mates, excessive grooming) the animals will be singly

housed with advice sought from animal care and welfare staff for the use of additional enrichment as required .

Our modern animal facilities conform to the Home Office codes of practice and are independently assessed and accredited.

Veterinarians will be available to give advice on care for the animals and can be contacted outside normal working hours if necessary by scientists and animal care staff

PROJECT 236

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	STRUCTURE AND ECOLOGY OF WILD BIRD POPULATIONS
Key Words	Ecology, Environment, Populations, Genetics, Birds
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The research aim is to understand the structure, ecology and population dynamics of wild bird populations in relation to environmental variation. Specifically, I aim to understand how population responses to environmental change reflect the pattern, magnitude and causes of phenotypic and genetic variation within and among populations, subpopulations and individual population members.

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 benefits of this project will comprise major new insights in population and evolutionary ecology, including the magnitude and causes of small-scale spatial genetic structure, large-scale population ecology and connectivity, and population dynamic and evolutionary responses to environmental change. This research will contribute to the development of efficient and appropriate management strategies for wild animal populations of conservation or economic importance.

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

Maximum of 2500 individual wild birds over 5 years.

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

The work will involve free-living birds. We will capture and ring individuals of target species, following standard British Trust for Ornithology (BTO) procedures. Small blood or feather samples will be taken from selected individuals, and logging devices may also be fitted, again following BTO procedures. All captured individuals will be

released back into the wild at the place of capture, following the short required period of handling (typically <<60 minutes). The level of severity is very low, and no or very minor adverse effects are expected.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The research aim is to understand the structure and ecology of wild bird populations, and thereby inform conservation and population management. These objectives cannot be achieved without using wild animals, since it is such that are the focus of the research.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used, and the exact individuals to be used, will constitute the minimum number of individuals required to ensure sufficient spatial, temporal, multi-generational and total coverage to answer the questions at hand with acceptable statistical confidence.

Refinement

Explain the choice of animals 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.

Refinement

Overall, the work will be refined through a combination of carefully selected catching methods and conditions, and implementation of required procedures on selected focal individuals by trained and experienced personnel.

PROJECT 237

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	The mechanisms of energy homeostasis regulation
Key Words	Obesity, Diabetes, Metabolism, Feeding, Behaviour
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Obesity and its associated diseases such as diabetes, cardiovascular disease and cancer are major health issues worldwide. Being overweight or obese will effect a majority of people in the developed world but unfortunately treatments for these conditions are generally not effective at preventing or treating obesity. Obesity results from an imbalance between energy (food) intake and energy expenditure A wealth of data from animal models and human genetics show that feeding is primarily controlled by the brain and a range of signals produced by other tissues which together also control the body's other metabolic functions. While we now have a basic understanding of the wiring of the parts of the brain involved in regulating feeding and metabolism in part through our own work in mouse models, it is clear that these mechanisms show great complexity and that a range of genetic and environmental factors are involved. It is also clear our lack of detailed knowledge of these mechanisms significantly hampers our ability to develop effective treatments and preventative strategies. The aims of the project are to continue to study the signals and brain regions that control feeding using mice and a range of approaches including those that allow the precise control of nerve activity. These studies will define the brain pathways involved in feeding and metabolism and reveal how diseases such as obesity and diabetes develop.

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)?

Increased understanding of the causes of obesity and diabetes may lead to the development of new therapeutic approaches to these conditions through discovery of new targets and pathways controlling metabolism. Treating obesity will then also hopefully lead to a decrease in its associated diseases, such as diabetes cardiovascular disease and cancer.

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

We will study mice and expect to use about 12500 mice per year over a 5-year timescale.

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

The overall level of severity of this licence will be moderate. However, most experimental animals will only undertake mild procedures, such as food intake and basic metabolic and behavioural phenotyping which have only minor adverse effects, such as weight loss from dietary manipulation and transient pain after systemic injection. The remaining animals will undergo metabolic and behavioural phenotyping which will be of moderate severity and this will include a number of surgical procedures which will be performed under general anaesthesia with analgesia post-operatively. Animals will be killed using a schedule 1 method or another approved method at the end of each study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The regulation of complex behaviours and metabolism involve the interplay between the nervous system, a number of tissues such as fat, liver, gut and muscle and the endocrine system which includes circulating hormones. Therefore, to model this physiological system requires an intact living organism in which all these systems are operative. Furthermore, behaviours such as feeding can only be measured in the whole animal. As we are ultimately interested in how these processes are controlled in humans we need to use a mammalian model for these studies

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Many of our protocols are designed so that we obtain the maximum possible data from a single animal. When breeding genetically modified mice we use strategies to maximise the use of offspring where possible. We use cryopreservation to minimise stocks of mice. All studies are designed with careful statistical considerations with respect to sample size, utilise strategies to minimize bias such as blinding and

randomisation and involve precise and reproducible assays which together ensure that the information we gain is robust while using the minimum number of animals.

Refinement

Explain the choice of animals 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.

Refinement

We will use the mouse. This organism represents the lowest mammalian species in terms of neurophysiological sensitivity for which both the genetic alterations which underpin the proposed studies can be fully implemented and which displays physiological and pathophysiological features seen in humans. In the studies of metabolism and obesity mouse models have proved in many cases to be excellent models for the understanding of both human physiology and disease. All the procedures are classified as either mild or moderate and will be performed under local, general or terminal anaesthesia when appropriate and with analgesia when necessary. We will utilise the most refined technical approaches to minimise welfare costs.

PROJECT 238

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	In Vivo Imaging in Alzheimer's Disease Models
Key Words	Imaging, Alzheimer's, diagnosis, therapy
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Alzheimer's disease (AD) is one of the leading causes of dementia amongst the elderly, characterised by increasingly worsening symptoms of memory loss, mood swings and problems with communications and reasoning. There is currently no objective physical measure for diagnosis of AD in living humans; physical/biochemical measurements to confirm diagnosis can only be done post mortem. This project aims to develop non-invasive imaging methods and agents to identify and monitor AD in living humans, The programme spans 'bench to bedside', covering a variety of novel imaging agents and methods, including combinations of imaging modalities.

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 benefits are that patients will have greater access to new and improved non-invasive imaging techniques and agents to enhance their clinical management; e.g. to see if plaques or other markers of AD are present, predict whether a treatment strategy will work (thus avoiding debilitating and expensive disease management in patients who will not benefit) or monitor whether a medical intervention is slowing down disease progression. Also the new methods will have applications to clinical trials, to measure the effectiveness of new drugs. New imaging methods developed will refine future animal imaging experiments, improving the quality and quantity of data per animal.

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

Mice (3500) will be used 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 levels of severity? What will happen to the animals at the end?

No adverse events are expected to be caused by the imaging methods used. Any adverse events expected are related to induction and maintenance of anaesthesia (animals may die from respiratory depression <1-2% and/or hypothermia). Mild discomfort may ensue from injection of substances (however, where possible, this is generally done under anaesthesia) or withholding of food prior to imaging (as done in the clinic) but efforts are being made to optimise anaesthesia, administration of substances and avoid unexpected adverse effects and/or deaths. Adverse events may occur as the animal's age which are related to the genetic alterations which mimic human disease (weight loss, drop in body temperature, reduced mobility, un-groomed coat) but careful monitoring will minimise as much as possible, any pain, suffering and distress. All animals will be humanely killed at the end of the experiments and tissues/blood taken for further analysis. However, if at any point during the studies the animals reach a predetermined end-point at which pain, suffering and distress can be avoided or minimised, then these animal will be humanely killed and tissues will be recovered for further studies.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Animals have to be used because 1) data generated from this body of work can inform whether to go forward to human clinical trials, 2) to validate mode of action of novel compounds, experiments are required which cannot be conducted in humans for ethical scientific reasons, 3) biodistribution in whole organisms (i.e. tracking the injected agents route/accumulation and excretion through the body), with intact biological barriers and excretion mechanisms, is key to clinical use. Non-animal alternatives cannot replace the complexities of the interactions of these probes in whole body systems or with realistic models of Alzheimer's disease.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Preliminary screening (e.g. testing novel substances/imaging probes with cells or tissues in a controlled environment in the laboratory) will eliminate agents/substances that are unlikely to be effective/useful in a living animal. This 'pre-

screening' means that only those agents that show promising results will progress to use in animal tests. This reduces overall animal use.

The use of imaging to determine biodistribution of novel contrast agents rather than conventional killing at sequential time points, with removal of tissues for analysis is a major contributor to reduction of numbers. Imaging allows repeated observations/measurements over a period of time (longitudinal study) on the same animal, with humane killing only at the last time-point. Thus, if a longitudinal study involves six time-points, the numbers of animals are reduced to one sixth by use of repeated imaging. Since each animal serves as its own control to compare different time-points, the data obtained are statistically more robust (reduction), requiring fewer animals. Moreover, distribution of contrast agent within organs, not just between organs, is obtained, and unexpected uptakes that may not be detected by conventional methods can be found by whole body scanning. All these attributes of imaging contribute to a greatly improved benefit : cost ratio (benefit = data quality and quantity, cost = animal numbers, procedures and their severity).

Refinement

Explain the choice of animals 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.

Refinement

Species: Mice are the species of the lowest neurophysiological sensitivity that provide the necessary size compatible with the scale of resolution or movement associated with the techniques being studies. Resolution of the whole body imaging techniques is of the order of 0.5 – 1mm. Distribution within smaller animals will be beyond these limits.

Pilot studies are small experimental groups which help us to decide quickly how best to design a statistically and scientifically valid experiment. Thereby helping develop better larger study design and reduce possible suffering. Generally, inhalation anaesthesia will be used to minimise transient pain or distress and where possible, used for blood sampling, contrast injection, weighing and combined with imaging techniques where it is mainly used for restraint. In addition, there would be full and complete recovery between periods of anaesthesia and/or food withdrawal; rehydrating of animals during long imaging sessions; monitoring of respiration and cardiac function and maintenance of body temperature during imaging. These steps will maximise the animal’s wellbeing whilst on a study. However, development of Alzheimer’s and Alzheimer’s like disease will inherently make an animal more susceptible to confusion and distress during ageing and also possible accompanying side-effects due to their inherent mutations. Therefore careful monitoring of animal

wellbeing, pain relief and analgesia will be used as required, together with veterinarian advice, to minimise any pain and suffering which may arise.

PROJECT 239

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Retinal ganglion cells: when, how much and how do they contribute to the design and function of the visual system?
Key Words	retina, retinal ganglion cells, visual development
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:
No	(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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

This project aims to improve our understanding of the way in which the visual system develops in early life, particularly the development of the way in which details of the image formed on the retina at the back of the eye are transmitted to the brain.

The project will focus on retinal ganglion cells (RGCs), the only cells connecting the eye to the brain. Our visual world is compressed into electrical impulses generated in the RGCs, and these signals are sent to the brain via the optic nerve. RGCs come in an incredible functional variety. There are 1 million RGCs in the human retina (45,000 in mouse), grouped into different functional classes. Each class conveys information about a different feature of the image (e.g. how bright it is, contrast, sensitivity to movement and direction and/or orientation of the stimulus, colour), forming thousands of parallel information channels. This is what we call “the retinal code”, allowing us to recognize and interpret the complex images that form our visual world.

During early life, around the time of birth, RGCs also play a crucial role in guiding the formation of connectivity throughout the visual system, both at the level of the retina as well as in brain visual areas that receive a direct input from RGCs. Immature RGCs have unique features: (1) they are spontaneously active, generating bursts of impulses that occur simultaneously in neighbouring RGCs, resulting in waves of activity sweeping across the RGC layer in the perinatal retina; (2) RGCs undergo massive naturally occurring perinatal cell death (PCD), or apoptosis (a very common phenomenon in various parts of the developing central nervous system), resulting in over 70% of RGCs dying before reaching adulthood.

In this project, we will investigate the role of retinal waves and PCD in determining how the RGC population impacts on the maturation of visual function. In addition, we will also investigate how different functional types of RGCs (some responding when the light is turned on, or when it is turned off, or when visual stimuli move in a particular direction, or are presented at a certain orientation) contribute to the retinal code.

Our work will use a combination of pharmacogenetics, *in vitro* and *in vivo* electrophysiology, neuroanatomy, behaviour and mathematical models to address these important questions.

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

This project will offer unique opportunities to investigate fundamental open questions about how the strategy these cells use to communicate determines how vision develops in the mammalian visual system. It will advance our understanding of RGC functional diversity in the mature retina, and how early-life potential for changes (plasticity) impacts on RGC functional development. The importance of retinal waves in guiding the development of visual connectivity is well established, but we will go an important step further by manipulating RGC activity levels in a reversible manner during distinct periods of development after birth, without the complications of permanent plastic changes normally arising from genetic manipulations. This will enable us to draw firm conclusions about how critical it is for RGCs to be spontaneously active and generate waves of activity during specific periods in early life in order to allow healthy maturation of the visual system. These experiments will clarify which aspects of visual development are more prone to irreversible modifications in adulthood (leading to disorders like amblyopia, or blurred vision, a common visual disorder known to have its roots in early development). Our project will also clarify for the very first time to what extent the size/density of the overall RGC population determines our visual performance (e.g. our ability to discriminate details) in adulthood. These experiments will provide important new knowledge for regenerative medicine (design of retinal prosthetic devices, stem cell therapy), by determining the optimal cell/electrode density required to restore vision, and more generally, by paving the way to design more efficient therapies for neurological disorders linked to changes in cell number (e.g. Down syndrome, autism) based on stem cell repair or neural prosthetics. Finally, our studies on understanding how different RGC classes contribute to the retinal code will provide a useful novel, global method to study cell diversity and classification and could be applied to other systems where cell diversity is key to understanding functional complexity.

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

The project will be done entirely on genetically modified mouse lines. Most mice we will use will have conditional gene expression in specific retinal cell classes, which

means that the mutation will not be expressed in any other cell in the organism except in these specific cell subclasses. We expect to breed about 1200 mice in total over a period of 5 years, enabling us to have sufficient animals with the appropriate combination of gene expression (400-500 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?

We expect our experiments to cause very minor adverse effects. None of the mouse lines we will generate are expected to look or behave any differently to normal mice, or to experience any adverse effects as a result of the genetic manipulation. Most of the procedures are classified as mild. The only procedure classified as “moderate” is intraocular injections of drugs or anatomical tracers. These will be done under general anaesthesia, and animals will be given analgesics to ensure they do not suffer pain and discomfort. They will be monitored during their recovery from anaesthesia and checked on at least a daily basis by the animal facility technicians for possible complications such as post-operative infections, in which case they will be given appropriate antibiotic treatment. Some other animals will be killed by Schedule 1 to isolate the retina for in vitro electrophysiology. Some experiments will end in procedures under anaesthesia with no recovery. These include animals used for in vivo electrophysiology and animals that will be humanely killed following intraocular injections of anatomical tracers to retrieve the brain and retina for anatomical studies.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

To understand an organ as complex as the brain or the eye, how it develops and functions in health and disease, there is no alternative to studying that organ in a living animal. Therefore, the use of intact animals is essential, particularly in the investigation of the senses such as vision. Moreover, we are particularly interested in understanding basic principles of neural wiring during development, and the best approach to reach this goal is to modify normal development, and this can only be done in intact animals. Alternatives such as cell culture do not allow access to the working sensory system.

Computational approaches are useful to work in synergy with experimental work, but they cannot replace it because we do not know enough about biological processes to build realistic computer models. Models can at most help us to refine our knowledge and understanding in an iterative manner. We have established collaborative work

with several theoretical neuroscientists and we will put all our experimental data at their disposal to develop biologically realistic models.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will use electrophysiological approaches to record electrical activity from RGCs (*in vitro*) and from neurons in the brain visual areas *(in vivo*). For *in vitro* experiments, we will remove the retina from freshly killed mice and place it on an array of electrodes that will record the activity from RGCs. For *in vivo* experiments, we will insert electrodes into specific areas of the brain that receive input from the retina. This will be done in anaesthetised mice. Our electrophysiological approaches, both *in vitro* and *in vivo*, provide a very high data yield per experiment. Indeed, we use large arrays of electrodes, allowing us to record from thousands of cells in each experiment. This approach allows us to drastically reduce the number of experiments required to achieve results with robust statistical significance.

Wherever possible, we will reduce the number of animals by performing behavioural testing before we use them for *in vitro* or *in vivo* recordings or anatomical tracing.

We routinely analyse data soon after each experiment. This allows us to see trends emerging from our results, helping us to reduce and refine our experimental work, and to stop gathering data once our results reach statistical significance.

Refinement

Explain the choice of animals 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.

Refinemen

The reason for choosing mouse lines in this project is first of all because it is a mammalian species, hence closer to humans than amphibians, reptiles or birds. It is widely used in vision research, and especially in developmental studies. Indeed, pups are very immature in terms of visual function at birth (they are completely blind), and the most important steps towards maturation occur during the first 2-3 postnatal weeks. There is tremendous potential plasticity during that relatively short period, allowing us to undertake many experimental permutations that can potentially result in permanent modification of visual connectivity and function in the adult, which is precisely the goal of this project.

Except for some control experiments using wild type mice, all all the models we have chosen are genetically modified, most of them with conditional gene expression in

specific cell types. This is one of the great strengths of our programme. Indeed, the models we have chosen will allow us to specifically silence early spontaneous activity (waves) at will (during distinct postnatal periods controlled by us) in most RGCs (but not in other cell types), or to modify the amount of PCD in the RGC population, yielding adult animals with modified visual connectivity and/or with either more or fewer RGCs. These manipulations will shed new light on the role of important early developmental events that are known to determine both structure and function in the mature visual system.

Animal suffering is minimised by using anaesthetics for protocols whenever necessary. Surgery for recovery procedures will be carried out in consultation with a veterinary surgeon. Any animal that exhibits untreatable signs of pain or distress during recovery will be humanely killed.

PROJECT 240

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Reproduction Safety Tests on Industrial and Agricultural Chemicals
Key Words	Toxicology, Reproduction, Safety
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The purpose of this project licence is to establish toxicological and safety data in animals following exposure to industrial and/or agricultural chemicals that Man may be exposed to. These studies performed are a regulatory requirement for sucessful market authorisation.

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)?

Chemicals play an important role in daily life. Therefore, their safety for Man, other animals and the environment has to be considered carefully. By establishing sufficient toxicological and other safety data in animals, safe handling precautions may be determined thus protecting the health and welfare of hundreds (e.g. for a site limited industrial chemical intermediate with limited potential for human exposure) to millions (e.g. industrial or agricultural product with world-wide market) of humans and animal species which may contact the materials concerned and facilitate the world-wide marketing and safe use of products. The projects performed under this licence provide safety data to facilitate sound regulatory decisions worldwide that protect the public and the environment from possible hazards. The regulated products have the potential to improve and enhance the health, well-being and quality of life of people and animals. For example, improved crop-protection increases food security, while development of safer chemicals and chemicals with reduced environmental impact is clearly beneficial for human and animal health and in environmental protection. The projects undertaken use methodologies that are well established and known to produce accurate and reliable results that can be used in regulatory risk assessment. Furthermore, the studies can rapidly identify any overt toxicity which would cease the development of the test item and therefore enable the Sponsor to make a decision at

the earliest opportunity to cease production: reducing the risk of possible human exposure and avoiding unnecessary expenditure and use of resources. The work performed under this licence will be undertaken in a GLP compliant laboratory thereby ensuring data integrity and accuracy.

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

The studies performed will typically use the rat and rabbit, Where the rat is deemed not to be the most appropriate species the mouse will be considered. It is expected that approximately 10000 mice, 17000 rats and 2700 rabbits will be used over a five year period.

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?

All animals will be dosed with the test chemical via a route that would mimic accidental exposure in man, typically in food, water or contact with skin at normally three dose levels and monitored closely for signs of toxicity. The majority of animals will have no clinical effect, with approximately 25% expected to lose weight or not gain weight at the expected rate, with only a minority (<5 %) exhibiting clinical signs of toxicity such as prolonged diarrhoea, tremors etc which necessitate immediate killing to protect animal welfare. The administration of the test chemicals to a pregnant animal may cause unborn foetuses to be affected which may include foetal abnormalities which result in death in the uterus. Some study designs require assessments on the development and behaviour of the adults. Assessments include the monitoring of activity and behaviour and testing of an animals grip strength, these test cause minimal discomfort only. All procedures performed on the animals are fully validated and established within the industry to cause the minimum distress and will only be undertaken by trained staff. On completion of each study the animals will be humanely killed and a post-mortem performed in order to establish effects on organs and tissues which aid the evaluation of the toxicity of the chemicals.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

It is generally accepted that the way in which a material is metabolised and distributed within a living body has a significant effect on how it works and its potential toxicity. In addition, effects on complex interacting biological systems cannot yet be replicated in in-vitro or ex-vivo tests. Consequently, for the majority of chemicals it is imperative they are tested on living animals in order to assess for

toxicity to tissue, organs and systems e.g. the cardiovascular, respiratory and reproductive systems following repeated exposure.

As the use of alternative methods, including the use of dead animals cannot, at this moment in time generate relevant data which supports the submission of safety data to international regulators, alternative methods such as in-vitro techniques will be used as much as practicable to supplement the work involving protected animals.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The number of animals used will comply with the requirements of the relevant regulatory guidelines and will be the minimum practicable to achieve the objectives of the study and allow meaningful interpretation of the data. The definitive number of animals required depends on whether or not the group is expected to demonstrate an effect. For a high frequency effect fewer animals are required, to presume the absence of an effect the number required varies according to the endpoint being considered, its prevalence in control populations or dispersion around the central tendency.

For all but the rarest events such as malformations, and total litter loss, evaluation of between 16 and 20 litters per group for rodents and rabbits tend to provide a degree of consistency between studies. Where there is a steep dose response, or blood samples are needed for toxicokinetic purposes for example, then additional groups may be necessary.

Sequani has significant experience running reproduction toxicology studies and the knowledge gained will be used to design studies capable of achieving their objectives.

Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of the studies to be performed, with power-sample size calculations performed for specific studies if necessary to determine group size. For preliminary studies, small groups are acceptable because of the use of overt toxicological endpoints. Where group sizes are sufficient (rodent studies), data from definitive toxicity studies are analysed statistically.

Refinement

Explain the choice of animals 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.

Refinement

It is generally desirable to use the same species and strain as in other toxicological studies. Reasons for using rats as the predominant rodent species are practicality, comparability with other results obtained in this species and the large amount of background knowledge accumulated.

In the majority of cases outbred rodents and the New Zealand White Rabbit will be used as these strains have good fertility and provide sufficient foetues/litter sizes for the assessment of test item related findings.

In embryo-foetal toxicity studies only, a second mammalian species traditionally has been required; the rabbit being the preferred choice as a non-rodent species. Reasons for using rabbits in embryo-foetal toxicity studies include the extensive background knowledge that has accumulated, as well as availability and practicality. Where the rabbit is unsuitable for example they do not show exposure, a second rodent species, for example the mouse may be acceptable and should be considered on a case by case basis.

Regulatory authorities require characterisation of toxicity at the Maximum Tolerated Dose (MTD) to ensure robust evaluation of safety before potential human exposure; it is therefore, necessary to perform toxicity studies at high doses that produce overt toxicity, usually in terms of clinical signs or body weight loss or depression of weight gain against age-matched controls. Response to observed effects will depend on the objective of the study; on preliminary studies where the objective is to determine the MTD then doses will be increased until effects are evident; once clinical signs or extent of weight loss indicates that a dose is unsuitable for use on a definitive study then action would be taken to alleviate the clinical signs, usually this would involve termination of the sex/group or may involve reduction of the dose.

Definitive studies should also show evidence of toxicity and typically clinical signs or reduced body weight gain would be seen in the high dose animals following dosing: some clinical signs may be present for up to 8 hours after dosing but would be expected to show clear signs of recovery at this time. Generally clinical signs would be expected to be absent prior to dosing on the subsequent day although some animals may still show some moderate clinical signs . Clinical signs will generally be expected to be up to moderate in severity . However, because toxicity can become worse with increased duration of dosing or the exposure of animals to the test substance may increase over time, sometimes high dose animals and occasionally lower dose animals may show significant adverse effects and in this case action will be taken to alleviate the clinical signs such as withdrawal of the animal from dose, reducing the dose (if appropriate) or termination. In some cases, effects seen on definitive studies can differ from those seen on preliminary studies for no apparent reason. A single clinical sign is generally unlikely to be sufficient to warrant termination unless the presentation is severe and the combined effects of multiple

signs will always be assessed for cumulative harm to the animal . Preliminary studies when performed will allow the delinitation of appropriate intervention and humane end points. Definitive studies will be performed within a moderate severity limit, as the use of humane endpoints, careful monitoring and rapid response to observed effects will negate the need for a severe limit.

Where little is known about a substance, or a class of substances, before commencing preliminary studies consideration will be given to using staggered starts where the effects in one group are assessed before commencement of dosing groups at higher doses. In all cases, dose selection will be based on all available information; data from preliminary studies or in some cases, and data from published literature.

PROJECT 241

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Cortico-thalamo-cortical pathways in rodent cognition
Key Words	Thalamus, Cortex, Learning and memory, Schizophrenia, Decision-making, Navigation
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

Brain studies related to the sensory systems show that interactions between the thalamus and cortex provide ongoing, dynamic feedforward and feedback lines of communication. My research focuses on understand how and when cortico-thalamo-cortical interactions linked to the dorsal thalamus are important for cognitive functions. Previous evidence in animal models indicates mediodorsal thalamus and cortex interact together during daily learning and adaptive decision-making, while anterior thalamus and cortex interact together during spatial navigation and memory. This evidence shows that these interactions are dynamic and ongoing, with thalamus and cortex being necessary partners in brain functions that are important for successful cognition. However, as yet we do not understand how the thalamus and cortex interact together and how neurotransmission of signals is relayed between these structures for successful cognitive functions to occur. This research will address these questions.

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)?

Neuroimaging and neuropathology studies show that links between the cortex and dorsal thalamus are altered in the human population with schizophrenia, dementia, Parkinson’s disease, major depression and other mood disorders. My work on the mediodorsal thalamus and cortex in animal models provides invaluable insights related to understanding the causal neuronal influences leading to the cognitive symptoms associated with these neuropsychiatric disorders and normal healthy ageing. Furthering research in animals, in addition to studies in patients and healthy controls, is an extremely fruitful way to gain greater fundamental knowledge about how the brain works, and advance our understanding about the neural mechanisms

that provide the neurotransmission of brains signals important for cognitive processes to occur.

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

Rats and mice (including genetically altered mice). Over the course of the 5 years we expect to breed in the region of 1,000 mice. Mice (150) and rats (1425) will be used in the Protocols. Each specific experiment runs for a shorter duration (up to 18 months) over the course of the 5 year project licence.

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

There will be genetically modified animals bred on this licence. However, the genotypes are not anticipated to impact on the animal's welfare. Most of the genetically modified mice and wild type mice, as well as some of the wild type rats will be used for neuroanatomy studies in which they will experience recovery neurosurgery under general anaesthesia to inject neuroanatomical tracers into targeted brain regions to determine their interconnected brain structures. Some (up to 15%) of these animals (genetically modified mice, and wild type rats and mice) will receive in vivo electrophysiological recordings to identify a specific neuron of interest combined with injection of tracers to that specific neuron under terminal general anaesthesia. The remaining wild type rats will learn behavioural and cognitive tasks and will receive either, micro-lesions of brain structures, or injections of drugs that target specific brain receptors combined with implantation of subcutaneous mini-pumps to determine their impact on cognitive and behavioural testing. Other animals will also learn behavioural and cognitive tasks, and via their implanted devices, they will receive injections of neurochemicals to temporarily disrupt neural activity into discrete targeted brain regions to establish their causal influence while performing the tasks. Other animals will also learn behavioural and cognitive tasks, and/ or receive discrete brain lesions or neurochemical inactivations of brain structures and receive implants to allow electrophysiological recordings of neurons in the brain to establish, what signals are conveyed and when across brain netowrks during cognitive and behavioural testing. For the animals with lesions, the damage to the brain will only produce impairments associated with subtle cognitive deficits. If any motor, visual or other sensory system deficits were observed in the animals then they would be terminated because these effects would compound the science by distorting the results of the experiments. The principal adverse effects are likely to be associated with be the surgical procedures for injection of inert tracers, or creating small lesions into the brain, or the injection of drugs that target specific brain receptors combined with implantation of subcutaneous mini-pumps, or the attachment to the skull of chambers for neuronal recordings, or of chronically dwelling cannulae positioned appropriately for later intracranial administration of drugs. Some animals will also receive systemic administration of substances. Some

animals will have their food intake restricted to facilitate their responding for food rewards in the appetitively motivated tasks. Any pain, suffering distress or lasting harm will be carefully monitored and relieved by appropriately administered drugs by our very experienced veterinary team, led by the NVS and the NAWCO, PILs and PPL holder. The level of severity is moderate. We work in close contact with the NVS and NACWO to continually assess the impact of the procedures on each individual animal. The animals are killed by a Schedule 1 method or are deeply anaesthetised and killed for perfused at the end of their experiments so we can assess the changes in their brains and document the neuronal tracts that connect these regions together in further ex vivo detailed histology studies.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In this research, we are studying how the brain is put together and how individual nerve cells contribute to the networks of nerve cells in the brain. Animal usage is necessary for this project, because invasive *in vivo* methods are currently the only way to study actual real-time brain mechanisms and establish their causal influence.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We will ensure that we use the minimum number of animals:

- by careful monitoring of the breeding programme to ensure that we do not breed excessive numbers of animals

- by use of our multidisciplinary approach that allows the maximum amount of data from individual animals

- by good experimental design, the application of the most appropriate statistical analyses and regular consultation with other senior colleagues and the University statistical services.

Refinement

Explain the choice of animals 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.

Refinement

We use animal models to study the pathway interactions of the dorsal thalamus and the interconnected cortex and other subcortical structures. For these particular studies involving neuroanatomical investigations of brain neural networks, or cognitive and behavioural assessments after alterations to brain development, or selective drug manipulations of these pathways, mice and rats for the neuroanatomy and rats for the behavioural and cognitive assessment studies are the most appropriate animal model.

We minimize welfare costs to the animals by the highest standard in animal husbandry; the highest standards in surgical procedures and peri-operative care that is equivalent to the highest veterinary standards.

PROJECT 242

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Regulatory Testing of Biological Medicinal Products
Key Words	Biosafety
Expected duration of the project	5 year(s) 0 months

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

Purpose	
No	(a) basic research;
	(b) translational or applied research with one of the following aims:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or

	products, with one of the aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall aim of this project licence is to conduct Biosafety studies with biological medicinal products (for human and veterinary use), and to produce reagents and experimental materials for regulatory, diagnostic and research purposes. The specific objectives include:

To conduct safety and potency studies to support the licensing of novel human and veterinary medicinal products such as flu and measles vaccines .

To produce polyclonal antiserum, required for safety and potency studies mentioned above.

To produce Cryptosporidium oocysts.

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 are that Biological Medicinal Products (BMPs) can be tested and guaranteed to be safe for general use. These BMPs are mainly produced by novel biotechnological processes and include naturally occurring human proteins and hormones (e.g. insulin required by diabetics), blood products (e.g. factor VIII required by haemophiliacs) and cytokines (e.g. interferons used in the treatment of cancer). Novel viral vaccine antigens (e.g. Ebola) produced by recombinant DNA technology are also tested and all these products are undoubtedly having a profound impact across the entire medical spectrum. Many of these BMPs have added treatment options for diseases which previously had no treatment options. There are however potential safety concerns that arise from the novel processes used in their manufacture and from the complex structural and biological characteristics of the products. Such products therefore require robust testing by in-vitro and in-vivo methods to allow adequate assessment of safety. A risk based approach is

commonly taken by regulators depending on the product itself and the manufacturing process. So it is essentially because the products are novel that the robust testing schedule is required (or at least until there is more historical data to confirm that a type of product or a production process is safe). Cryptosporidium is a parasite found in the environment which, if ingested, can cause severe illness. The parasite can only be produced by means of experimental ovine/bovine models (and purified from faeces) and the shelf life is not more than 3 months. Parasite stocks are required by the UK Water Boards for statutory testing of water and numbers of parasite required for this QC testing has been refined downwards over recent years meaning that the number of animals required is less than previously used.

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

Eggs - Approx 5,000 per year Mice - Approx 12,500 per year Guinea pigs - Approx 500 per year Hamsters - Approx 300 per year Rats - Approx 300 per year Rabbits - Approx 25 per year Cattle - Approx 10 per year Sheep - Approx 10 per year

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?

Adverse effects in safety and potency studies are few and infrequent therefore >99% of animals will complete a study without experiencing pain or discomfort except at the time of administration. The occasional animal experiencing pain or discomfort is normally due to toxicity of a medicinal product which the manufacturer was not anticipating. Animals are always euthanased at the end of a study by an approved method. Calves and lambs infected with Cryptosporidium are likely to experience diarrhoea and potentially stomach cramps. Intervention with electrolytes call alleviate any discomfort and animals may be returned to stock after a few days

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

In every instance, the safety of new products is being evaluated in animals to comply with regulatory requirements. It is therefore not possible to move towards *in vitro* alternatives without approval from governmental regulatory bodies.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

Studies will only be conducted when there is a reasoned, sustainable justification for the generation of new test data, there is no validated alternative to animal tests, and the protocols will produce data that will meet the specified objective, will be scientifically valid and will be acceptable to the relevant regulatory authorities.

Group sizes are set in the various regulatory guidelines and the criteria for a test ‘pass’ or ‘fail’ are clearly defined in the pharmacopoeial monographs.

Refinement

Explain the choice of animals 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.

Refinement

Before a study can commence, we ensure that the protocol cannot be refined further (although it must always be compliant to the relevant regulatory guideline).

We strive to keep up-to-date with regulatory developments by attending relevant conferences and, importantly, by maintaining on-going communication with regulatory bodies. One example of our effort to refine studies is the action taken when some regulatory bodies (in particular the US Regulatory Bodies) insist on the individual housing of animals when it would appear that there is no sound scientific rationale for such a requirement. Our Group's Experiments and Ethical Review Committee has written to national and international regulatory bodies to express its concern and we have received positive feedback from these confirming that those comments would be taken into account when discussing harmonised international guidance documents.

PROJECT 243

NON-TECHNICAL SUMMARY (NTS)

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This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Regulation of Energy Balance
Key Words	Calorie Restriction, Obesity, Body composition, Energetics
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;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

We currently face an obesity epidemic together with an increasing ageing population; obesity and ageing are two major risk factors for a number of life-threatening diseases such as cancer, type 2 diabetes and cardiovascular disease. Calorie restriction (CR; ie. dieting) is the primary self and physician prescribed treatment. However, successful weight loss is often short-term and long term dieting and sustainable weight loss appear to be unachievable to many. Maintenance of a steady body weight requires energy intake and energy expenditure to be in equilibrium. This is known as energy balance, which is a tightly regulated, complex system. The body is fine tuned to recognise when energy supply is low and when we diet the body preferentially uses energy from our fat stores to make up the negative energy imbalance, however, lean tissue is also utilised. Similarly, when an individuals' energy levels are in surplus both fat and muscle are stored. Changes in dietary composition ie. the source or amount of protein, carbohydrates or fat, also appear to alter the loss/ gain of body fat and lean tissues. While we know the coordination of energy balance is centrally controlled, we do not have a clear understanding on how the body recognises different dietary components. The aims of the project is to identify key genes and hormones involved in this response and ultimately design diets where specific macronutrients promote changes in body composition and link these changes to the to the key genes and hormones involved.

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 identification of key genes and diets that promote fat loss while maintaining lean mass will be of benefit to several public health related issues such as obesity and its co-morbidities (cancer and type 2 diabetes).

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

To address the highly variable response to dieting between individuals we will use mice as a model. Similar to humans the mouse also responds with varying degrees of weight loss (or gain) on a diet. Knock-out mice models may be generated from the identified genes which will allow the validation of their role in controlling energy balance. Aged mice up to 2 years may also be used for studies investigating the specific gene involvement in the life extending response to calorie restriction. Over the 5 year project licence we estimate the use of 3500 mice.

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?

CR is known to improve not just health but also lifespan, so although the mice will lose some weight this is not harmful. All dietary manipulations are tested for palatability before use. Measuring food intake can be done easily by weighing the food left each day. Animals will be handled and weighed daily. Some animals will need to be left to get old, but any animal experiencing age-related disease inflicting pain such as arthritis will be removed from the study. However to measure energy expenditure accurately we implant a transmitter. The surgery should not result in a greater than moderate severity, because pain is controlled by use of pre- and post-surgery pain killers and anti-inflammatory drugs. Once implanted transmitters are non-invasive and do not affect the activity or behaviour of the mice. Blood sampling will cause very short term needlestick pain. Administration of compounds affecting energy balance will be done via injection or the surgical implantation of small pumps subcutaneously, again using pain killers. In some instance these will be replaced once. To measure the changes in individual tissues, hormones and genes the mice are humanely culled at the end of study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

Where possible *in vitro* studies will follow up work from the animal research, ie specific response to compounds in muscle and fat cells can be done using cell culture.

However, is vital to understand how diet, hormones, genes interact and this cannot be done *in vitro.* Unfortunately there are no feasible alternatives to measure whole body response other than to use the mouse model.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

From 1 single mouse information on numerous levels are obtained from body weight to activity to gene expression levels. Study plans are rigorously designed to obtain the maximum information from the minimum number of animals. As part of the design we seek statistical advice to ensure animal numbers are adequate to meet statistical significance.

Refinement

Explain the choice of animals 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.

Refinement

While mice are the ideal model for looking at the phenotypic responses to manipulations of energy balance, we will use knockout models to ascertain the function of any genes of interest we identify. Any suffering throughout these protocols is minimised and appropriate analgesia and anaesthetics used where necessary. We have experienced staff and advice from NACWOs and NVS are sought where necessary. All animal procedures and analytical assays are designed to be as non-invasive as possible and the majority of our data are collected using non-regulated procedures. Where absolutely necessary surgical procedures are used with guidelines on aseptic techniques strictly adhered to. All techniques as well as the follow assays are refined to cause the least stress to the animals.

PROJECT 244

NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Central and peripheral control of growth and metabolism
Key Words	appetite, muscle, body composition, growth, metabolism
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:
No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

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

The overall *aim* of this programme of work is to increase our basic understanding of how our brains and muscles monitor the nutrition and metabolism in our body, and then use this information to regulate our appetite, our growth and our body weight. The experiments will look at three different systems. First we will explore how a recently discovered hormone (FGF21), that is produced by our liver and muscles signals when we have impaired nutrition, signals to other tissues including fat and the brain that we have low energy supplies. This will be done by treating fat and lean hamsters with this hormone, then putting anaesthetised animals in a scanner to measure how different tissues respond by taking up glucose or fatty acids. Second, we will investigate how tanycytes, a non-neuronal cell type in the brain, respond to changes in nutritional signals. We will examine whether they are important in sensing glucose and amino acids in the body by measuring appetite and body composition in mice that have been genetically engineered to produce less of these cells. We will also determine whether mice can sense these metabolic signals if nutrient sensing molecules are specifically removed from tanycytes. Third, we will investigate how muscles sense and take up amino acids to synthesise proteins. We have discovered a number of genes that increase expression when farm animals are treated with growth-promoting products, so will determine the real function of these genes by genetically modifying mice to express higher or lower levels specifically in muscles. Muscle growth and energy expenditure will be determined by keeping mice in metabolic cages, and by scanning them under anaesthesia.

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)?

Knowledge of how our nutritional and metabolic status is monitored, and how this information is processed to regulate appetite, growth and energy expenditure, and thus control muscle development and body weight is important for two reasons. First, the prevalence of obesity in both adults and adolescents in the UK and worldwide is still increasing. Not only does obesity and the associated metabolic syndrome compromise the physical and mental health and life expectancy of the individual, but it represents a major and increasing financial burden on society through the direct costs of healthcare, and through the indirect costs of reduced productivity. Understanding basic control systems in the body has the potential to identify therapeutic and interventional strategies to assist weight loss. We will be investigating the biological actions of a natural hormone fibroblast growth factor 21 (FGF21), closely related compounds are already undergoing evaluation for their potential in treating patients with type II diabetes by several pharmaceutical companies. Second, increasing world population and instability of economic systems has put increasing pressure on global food security. Understanding basic control systems of feed efficiency and muscle growth has the potential to identify mechanisms associated with enhanced efficiency and growth, such that specific cellular targets can be identified that should be amenable to dietary or therapeutic manipulation to enhance growth and therefore lean meat production.

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

Our studies will be carried out in laboratory mice or in Siberian hamsters. The control of appetite and energy metabolism is fundamentally similar in rodents and man, so using these less sentient species will provide important information. Mice are used because we have the technology to manipulate genetically genes of interest in this species. This will allow us to test specific hypotheses about the nutrient sensing mechanisms in tanycytes and muscle. We will carry out studies on FGF21 actions in hamsters because these rodents show a natural cycle of weight gain in summer, but then increased fat oxidation and weight loss in winter. We can induce these different energetic states simply by changing the daylength, so have an animal model where we can compare the effect of FGF21 in lean and fat states. We anticipate studying approximately 900 animals in total over the course of five years.

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

The core measurements that we make centre around determining food intake and energy expenditure when animals are removed from their home cages and placed in metabolic cages typically for 1-2 days. The level of severity is generally moderate because in some studies animals will have been placed under general anaesthesia such that microimplants releasing test substances can be placed subcutaneously, or so that guide tubes can be surgically implanted or infusions of gene constructs can be delivered directly into the brain. In studies where microCT scanning is used to

determine body composition and muscle size animals will also be placed under general anaesthesia, this is also a moderate severity procedure. In studies where a radiotracer is infused and PET scanning is used to monitor glucose or fatty acid uptake into tissues this is likely to be a terminal procedure under general anaesthetic. At the end of all studies animals are euthanised by a recognised schedule 1 procedure. Accurate monitoring of food intake is very sensitive to the health and wellbeing of experimental animals, so limiting adverse effects is paramount in experimental design. The likely adverse effects are largely short term, being related to discomfort after general anaesthesia and the surgical implantation of slow release implants or infusion of gene transfer vectors. Analgesic regimes will be agreed with our named veterinary surgeon to minimise this discomfort.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

The control of appetite and regulation of energy expenditure are aspects of behaviour and whole organism physiology of such complexity and diversity that they cannot yet be modelled by computer, nor can they be reduced to simple biological processes that are amenable to replacement with *in vitro* experimental approaches. The proposed objectives derive not only from previous in vivo studies, but also from in vitro and ex vivo approaches to get to the current state of knowledge. For all three objectives, in vivo studies are essential as a step in the translation of knowledge to human physiology and livestock production.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The underlying principles governing the numbers of animals used in specific experiments is that where sufficient preliminary data exists such that variation of response and magnitude of effect can be predicted, a power analysis will be undertaken to ensure that the study is sufficiently powered for the main experimental variable. Where such data are not available, for example in testing a previously unused compound or paradigm, then an incremental design will be followed. Typically with a novel compound, a dose-response approach would be taken where in a pilot study at most two animals are treated with a low dose, and pairs of animals treated with gradually increasing doses until a desired outcome is observed, at which point a fully powered study can be designed.

In order to ensure that high quality, reliable and valid data is extracted from the minimum number of experiments, the ARRIVE guidelines. The major experimental approach for collecting data is the use of a metabolic cage system. This allows the simultaneous collection of data on oxygen consumption, carbon dioxide production, locomotor activity, and meal size/frequency/duration, thereby substantially reducing the number of studies/animals that are required to meet the objectives. Studies are designed wherever possible such that multiple experimental measures are taken from the same individual animals, for example measuring metabolic and behavioural changes and then post mortem gene expression and endocrine responses to particular manipulations. This minimises the number of animals involved, and allow more powerful analyses by correlating and comparing responses on a within-animal basis.

Reduction of animal usage will also be achieved by using computerised tomography (microCT) to evaluate body composition, as an individual animal can be scanned on repeated occasions obviating the need to use separate cohorts of animals to assess body fat at different time points during a study. PET/SPECT will also be used to assess the impact of the various treatments on glucose (eg using ^{18}F-deoxyglucose) and fatty acid (eg using ^{18}F-palmitate) uptake. Likewise, microCT scanning will permit serial repeated assessment of muscle growth in individual mice rather than requiring multiple cohorts that are euthanized at different time points.

Refinement

Explain the choice of animals 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.

Refinement

The proposed studies will utilise rodents to address all the objectives because they are considered to be the least neurophysiologically sensitive mammals that nevertheless display fundamental mechanisms of hypothalamic control of energy balance that are comparable to and informative of those in man. The basic function of the hypothalamic and limbic systems involved in the regulation of appetite and energy expenditure are fundamentally similar in rodents and man, thus the use of rodents as an experimental approach is widely accepted; they are considered to be the least sentient mammals in which the objectives can be accomplished. The majority of procedures necessary to address the objectives of this licence are expected to fall in the mild category, but because some of the experiments require a period of general anaesthesia for either the surgical implantation of slow release subcutaneous implants or for placement of the animal in a microCT/PET scanner, then the overall severity would be moderate. Where surgical manipulations are to be carried out, analgesic protocols and aseptic procedures will be agreed with the NVS

in order to minimise any pain and to maximise the probability of a rapid and complete recovery.