NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Investigation of Cardiovascular Disease Mechanisms
Key Words	Heart failure, hypertension, diabetes, blood vessels
Expected duration of the project	5 years

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 aims to investigate mechanisms underlying development of adverse structural and functional changes in the cardiovascular system, known as remodelling, which is a major factor determining progression of cardiovascular disease. Cardiovascular disease currently affects ~7 million people in the United Kingdom and is linked with poor health, with the main causes being high blood pressure, diabetes and obesity. Despite improved treatments, cardiovascular disease remains the United Kingdom's biggest killer and is responsible for more than a quarter of all deaths. Therefore improved understanding of how cardiovascular disease develops is urgently needed in order to help in designing 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)?

This work will substantially increase our understanding of important mechanisms involved in the development and progression of cardiovascular disease in response to key stresses, including high blood pressure and diabetes. We anticipate that the knowledge gained from this research will be published in top journals for the benefit of other researchers and will help to identify new and more effective ways to treat cardiovascular disease in humans.

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

Our studies will use several animal models of cardiovascular disease which mimic common clinical conditions. Due to the availability of genetically-modified models, we will use mice and expect to use a maximum of 2,500 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?

All animals will be carefully monitored for adverse effects, such as weight loss and infection. Our models of cardiovascular disease may be associated with some unpredicted death (up to ~20% mainly due to acute heart failure), which is necessary to accurately reflect what happens in humans. Surgical implantation of a pump which releases a hormone to increase blood pressure in mice should not result in any visually apparent effects other than seeing the pump itself. Induction of heart attacks in mice may be associated with up to 20% unpredicted deaths up to one week after surgery, but in our hands is typically below 10%, occurring without visible preceding signs. Some leg pain on exertion and reduced movement is expected in mice subjected to a model which mimics poor blood flow as seen in e.g. smokers. However, this typically only lasts for a short time after surgery and is relieved by administration of painkillers as necessary.

The expected level of severity for most protocols is moderate or severe. However, all procedures have been established for many years and optimised to reduce animal suffering so we do not foresee additional adverse effects. At the end of experiments, all 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 it is useful to study individual pathways in cell models, their contribution to overall cardiovascular disease development, which involves many interacting factors and occurs over a longer time period than can be practically studied *in vitro.* This necessitates the use of animal models to accurately represent the complexity of disease processes which are much more usefully assessed in a complete organism.

All of the animal models which we will use are designed to accurately mimic human disease with the aim of helping to identify new treatments. As such, this work would be impossible to pursue without the use of animals.

Reduction

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

Reduction

The numbers predicted have been estimated by using power calculations which determine appropriate sample sizes based on previous studies and expected differences between experimental groups.

All efforts will be made to minimise the number of animals used. For example, cardiac function and blood pressure will be recorded by ultrasound and radio-telemetry, respectively, which allows repeated measurements in the same animal, and tissue for laboratory studies will be collected afterwards. Furthermore, control procedures will be reduced by designing experiments in such a way that several treatment groups are compared with a single reference group.

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 given appropriate anaesthesia and analgesia and clear criteria are stated in order to minimise suffering. If any animal shows signs of excessive discomfort or suffering then it will be immediately killed. All of the procedures that we will be using have been developed and refined over many years so that they expose animals to the minimum level of discomfort. For example, several of our surgical models are now minimally invasive which means that animals experience much less damage to the skin and tissue layers so make a quicker and more complete recovery. Our procedures are constantly reviewed and refined, incorporating new technology, so that harm to animals is kept to an absolute minimum.

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Safety and Efficacy of Drugs in Domestic Species
Key Words	Safety and efficacy
Expected duration of the project	5 years

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.
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 fulfil regulatory requirements with regards to the safety and efficacy profiles of potential drugs in the target species. Safety for consumers of food producing animals will also be investigated.

What 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 be involved in developing suitable formulations and bringing numerous drugs to market, across a range of species, thereby increasing the number of products available to the end-user to treat a wide range of illness/diseases in animals.

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

During the five year lifetime of the licence we expect to use up to:

Cattle 1720

Sheep 1620

Pigs 1620

Goats 390

Horses 290

Dogs 1000

Cats 1010

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 vets will confirm suitability for the study by a clinical examination and/or review of blood analysis results. Animals will be treated with a test and/or reference drug and the drug concentrations will be measured by sequential blood sampling to determine the safety / efficacy profile of the drug. Blood samples may also be taken to determine the effects of the drug on the animal. Therefore the animals will be required to be restrained which often causes stress, and be subjected to multiple needle sticks, as well as having the dosage administered, either orally, topically, or by injection which will cause short term-pain.

In a small number of studies, it may be necessary to individually house animals and it is acknowledged that this is a harm. Such harm will be mitigated as much as possible by refinement measures.

An acclimatisation period of no less than 4 days will be necessary to allow the animal to adapt to its surrounding, and to help reduce any stress caused by the experience.

Expected adverse events are predicted to be rare and generally mild, involving gastrointestinal signs (vomiting, diarrhoea) and inappetance. There may also be some bruising / swelling at point of injection sites which will be kept to a minimum by best practice.

Adverse events associated with blood sampling or catheterisation sites may include: infection or inflammation of the surrounding skin or inflammation of the catheterised vein. There may be bruising of the skin surrounding the site. Best practice will ensure these are kept to a minimum and twice daily monitoring will be conducted to identify any issues early.

Animals may be rehomed (all species) / sent for commercial slaughter (farm species) or re-used (all species) if deemed suitable by the Named Veterinary Surgeon (NVS).

Application of the 3Rs

Replacement

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

Replacement

Relevant guidelines require specific criteria be proven in each of the animal species, therefore the use of animal studies is unavoidable.

Where possible, the drugs may be developed using a different approach to show similarities between the drugs under test, avoiding the need for animal work.

Reduction

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

Reduction

There will be a number of steps taken e.g. testing to show the article maintain its quality, investigating rates of dissolving the tablets (where possible) before any animal studies commencing so as to remove from the animal testing any test drugs failing to meet specific criteria. These drugs will not then be tested on animals, hence minimising animal studies required.

Full literature review will maximise the findings from studies, thus reducing need for unnecessary repeat studies.

Early probe studies will be conducted in the minimum number of animals required to allow sound statistical conclusions. The results of these studies will also be used to predict the number of animals to be used for subsequent studies.

The number of studies to be conducted will be kept to the minimum required to permit the approval of the drug on the market.

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 model for each study type is as per regulatory guidance documents.

Animals will be monitored throughout the study by trained personnel, with a veterinary team available to advise on any additional course of action required.

Housing requirements will be as per Code of Practice (unless scientifically justified) and species specific enrichment will be provided.

Indwelling intravenous catheters can be used to facilitate blood sampling and reduce animal stress where multiple samples are required.

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Word limit; 1000 words

Project Title	Understanding Immunity to Prevent or Treat Infection
Key Words	Bacteria, infection, immunity, vaccine
Expected duration of the project	5 years

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);
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):

We are rapidly losing the ability to treat bacterial infections as they become more and more resistant to our current antibiotics. When this happens, people will die from infections and many of our current medical procedures (such as basic surgeries or chemotherapy) will become impossible due to the infection risk.

Within this project we are trying to understand how the body fights bacterial infections. We focus on the bacteria that the World Health Organisation has described the biggest threats. We aim to look at the parts of the bacteria that induce immunological memory and understand how these memory responses are able to kill the infections.

What 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 us to much better understand how our immune system defends us against bacterial infections. We will be able to use this information to help to develop new vaccines to prevent infection and potentially new medicines that can be used to help clear infection when they do occur.

As we develop a clearer understanding of the immune response to infection, we will share this information with other scientist and doctors via conferences and publications.

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

Within this project we expect to use approximately 17,400 mice over the course of 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 mice will be infected with bacteria; generally this will be put into their lungs. The animals will go on to develop lung infections (pneumonia) and feel unwell. Once they have been infected, we check the mice ever few hours, weigh them and record any symptoms.

The animals will be given injections which will make the immune cells either more or less active. Rather than waiting to see if this stops the mice dying, we end the experiments before the mice get really sick. We will then measure how many bacteria are in the lungs and the levels of immune molecules – this tells us how sick the mice were.

In general the symptoms the mouse will experience (generally feeling sick, breathlessness, shivery) are moderate. However, on occasion an animal may get sicker than we expect and it would have to be euthanized before then end of the experiment. Therefore, as this may occur, the overall severity of these infection protocols is severe.

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 use alternative methods to study these cells. We carry out experiments using human cells that we grow in the laboratory.

However, our bodies have multiple ways of fighting off infection. The bacteria also have may ways to try to avoid and overcome our defences. This complex battle between the two makes it almost impossible to model this in the laboratory.

We therefore need to use animal models to investigate how these immune cells work and how we can make them more effective at defending against bacterial infection.

Reduction

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

Reduction

We are committed to only using the number of animals that are absolutely required. When possible, we will infect the mice with bacteria which are bioluminescent (glow in the dark) – we can use a special camera to detect these bacteria inside the live mouse. This means we can measure bacteria numbers at several time points in the same animal, rather than having to kill mice at each time point and grow the bacteria from their lungs. We are also looking at new ways to monitor damage to the lung such as ultrasound and computed tomography scanning to minimise the number of animals 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

It is important to use a mammalian animal model to ensure that they have similar immune cells to humans. Mice are a good choice as their immune system has been very well studied and there are many methods available to investigate their response.

Additionally, their size is perfect for imaging bacteria. The glow from the bacteria can only travel through a few millimetres of animal tissue. In larger animals the lungs are too deep within the animal to be able to see the bacteria.

We try very hard to ensure that we minimise the discomfort the mice we use experimentally. They receive anaesthetic before we infect them to prevent the unpleasant feeling of fluid entering their lungs. Whilst they are anesthetised we apply gel to their eyes to prevent them becoming dry and sore. We also prop their heads up with beading ‘pillows’ this prevents them from having their faces in the sawdust and keeps their airways clear. We have also noted that whether or not the mouse makes a bed after it has been infected is a very good indicator of how sick it is feeling. When a mouse doesn’t make itself a little nest we monitor this animal more closely as we know it isn’t feeling well.

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Word limit; 1000 words

Project Title	Blood sampling of Chickens for Erythrocytes (Non Recovery)
Key Words	Blood Erythrocytes Chickens
Expected duration of the project	5 years

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);
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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 license is to supply biological reagents (i.e., chicken erythrocytes or red blood cells) that are required as a component of assays used for avian health screening and diagnosis of 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)?

Such serological testing enables monitoring of notifiable, zoonotic avian disease, thereby complying with animal health legislation. The use of the chicken erythrocytes allows for the rapid diagnosis of avian influenza epizootic disease outbreaks.

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

Up to 200 chickens are required per annum dependent on testing needs.

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 erythrocyte production will be carried out in strict accordance with standard operating procedures for the extraction of blood by cardiac puncture and use of anaesthetic in chickens and will only be carried out by licensed and fully trained staff members

The severity of the procedure is defined as non-recovery as the chickens do not regain consciousness. If the chickens display any symptoms of distress during the process, they are immediately administered an overdose of anaesthetic and subsequently killed by dislocation of the neck

Death for all birds involved in this procedure is ensured by final dislocation of the neck and leaving the birds for a minimum of two minutes to confirm that they are dead.

Application of the 3Rs

Replacement

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

Replacement

The requirement for the use of chicken red blood cells (erythrocytes) in the Haemagglutination Inhibition (HI) Test and Haemagglutination (HA) test is stipulated in the European Community (EC) directives and the World Organisation of Animal Health (WOAH) Manual for Standards for Diagnostic Tests and Vaccines.

Reduction

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

Reduction

The number of chickens used will be determined by the needs of the laboratory, however, it is estimated that the total number of chickens required for use per annum will not exceed 200. A minimum of 3 birds must be bled at any one time as the blood from these must be pooled for use in the assays as stipulated by WOAH .

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

At present chicken red blood cells used in the HI/HA tests is stipulated by EC directives and the WOAH Manual for Standards for Diagnostic Tests and Vaccines, there are no suitable alternatives available.

The chickens are housed and handled and cared for by dedicated fully trained staff. All procedures under this project license must be performed by staff that have successfully completed the animal licence handling course and have been fully trained in the required procedure.

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Word limit; 1000 words

Project Title	Novel Technologies for Cattle to Improve Sustainable Production
Key Words	Bovines, enteric methane, oxidation cap, trocar, cannula
Expected duration of the project	4 Years

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.
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;
Yes	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

Yes	(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):

The overall aim of this project is to validate a methane oxidation cap attached to an intraruminal trocar on performance, rumen function, methane emissions, welfare, and health of nonpregnant /dry dairy cattle.

Specific objectives:

1. To protect the environment by reducing methane emitted from rumen of dry cows.
2. To determine if there is a negative impact of inserting the device on health and welfare.
3. To determine if the device has a detrimental impact on animal performance

What 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 expected to include the following:

a) Reduce enteric methane emissions by 50% without affecting cattle performance and welfare.

b) This technology may be able to provide a novel tool for ruminant livestock industries to reduce the global methane load from all livestock systems. This technology, if approved, may have a long-term benefit; especially in the third world setting where the application of methane abatement practices is limited, and ruminant livestock are essential for food, work, soil health and environment diversity.

c) This research project has the potential for global importance to help prevent further global temperature rise.

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

8 dairy origin cows (nonpregnant and nonlactating) over a period of 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?

Within this study, the surgical insertion of the cannula device is a traumatic procedure and is expected a moderate level of severity; however, local anaesthetic and long-acting non-steroidal anti-inflammatories will be administered to the animals. Cows, in the rest of the procedures of this protocol, will suffer only mild discomfort as a result of collection of a range of biological samples, e.g. rumen fluid and blood.

However, if an animal shows post-operative infections or prolong signs of distress during a procedure (i.e., placed in respiration calorimeter chambers) may raise the level of severity to moderate. These events will be assessed by the Named Veterinary Surgeon (NVS) and treatment will be implemented as recommended, and, if required, removed from the study and euthanised.

At the end of the study, animals undertaking this protocol will be killed under Schedule 1.

Application of the 3Rs

Replacement

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

Replacement

The project will aim to complete the final testing of a device which will oxidise methane directly from the rumen of cattle. Much testing and modelling were done in a commercial company's laboratories as well as consultation with a university and research institution advisors in the United Kingdom who have a speciality in the topic of methane mitigation, extraction, ruminant physiology and welfare. Extensive literature reviews have also been undertaken in the development of the device. Development has also included work with postmortem animals to accurately ascertain the anatomical structures. However, the inter and intra animal variation in methane production is difficult to model in vitro. A commercial company has developed a machine that can mimic pressure and flow of methane based on a methane emission data set obtained from methane chamber work from a United Kingdom research institution and this has been used to develop the prototype methane oxidation device. However, in vitro development does not allow to add in the behaviour of the animal and how this will affect methane production and also the supply of methane to the oxidising cap when it is in-situ on the animal's flank, attached to a rumen trocar. Study on live animals is also now needed to determine the healing process following the insertion of the device, as well as its durability when exposed to normal housing and grazing conditions.

This is a novel technology to reduce emissions from enteric fermentation of ruminants and therefore testing on these species cannot be avoided.

Reduction

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

Reduction

Initial development work using fistulated cattle in respiration chambers, suggests that approximately 70% of all the methane in the rumen headspace gas can be extracted from a percutaneous rumen cannula device. In vitro work using a bottled gas mixture based on the concentrations in the rumen headspace and a machine that can model pressure and flow of the gas from the rumen suggests that 100% of all methane can be oxidised over a catalyst. With this as a priori data we have conservatively estimated the treatment effect of the oxidation device to be 50% of daily methane emission oxidised. Due to this large effect size, the number of animals required is small, with repeated measurements being more important than the total 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 methane oxidation cap and intraruminal trocar have been developed to account for the methane produced during the fermentation process of the diet in an adult bovine; therefore, an adult bovine is the most indicated model for this study.
For trocar surgery, perioperative anaesthesia will be provided by distal paravertebral infusion of local anaesthetics. Blocking of pain from the insertion site of the cannula will be tested with a needle prick, prior to the skin incision being made. Postoperative pain relief will be provided by way of non-steroidal anti-inflammatory administration. All the test animals will be monitored in the postoperative period for any complications of tissue healing around the cannula. Further treatment and pain relief will be provided if necessary. When the fistula wound is healed, the initial cannula (healing cannula) will be replaced by a refined cannula(s) that will adjust and avoid the stretching of the rumen fistula. The cannulas have a retention mechanism inside the rumen that opens and closes using the cannula applicator, this mechanism facilitates the cannulas exchange minimizing the animal's harm or distress.
Whenever the cows are in respiration chambers (3 or 6 days) tethering is necessary to ensure that animals do not break the crystal panels of the equipment. Wall are glass so they will have sight of neighbouring animals, as restricting visibility could cause discomfort and affect the accuracy of results. Whilst tethered, although not able to turnaround, cows will be able to stand up, lie down and feed/drink freely. Exiting the metabolic chambers, cows will re-enter the metabolic chambers not before a minimum of three weeks rest period since the last entry. Cows will be free of any tethering during the resting period, e.g., tethering in the byre. Each cow will enter the metabolic chambers no more than 10 times each year.
When a series of optional steps within a protocol are planned e.g. rumen sampling and blood sampling, these will be undertaken at the same time whenever possible, to reduce stress and discomfort due to handling.
We will ensure that all staff are fully trained in the implementation of the techniques being adopted and are alert to signs of suffering/distress within the animal.
Animals undergoing regulated procedures are checked by a veterinary surgeon. Staff are aware that any animal can be removed from an experiment at any time if its welfare is compromised.
Although the overall severity rating of the protocol is moderate, in isolation all the measurements proposed within the programme, except for tethering and surgery (moderate), are mild and provided they are undertaken by competent and trained staff, cause only minor/transient discomfort to the animal.

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Stimulus Responsive Therapy for Infection
Key Words	Antimicrobial infection, ultrasound, sonodynamic therapy
Expected duration of the project	2 Year 2 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):

Antimicrobial resistance (AMR) is currently recognised as one of the leading global health challenges of the 21st Century. It has been estimated that in 2019, antibacterial resistance was directly responsible for some 1.27 million deaths globally. Here we will develop a new approach based on the use of a drug that only exhibits antimicrobial activity when exposed to either light or ultrasound. However, two major challenges exist that hinder the approach. Firstly, infecting bacteria in tissues tend to grow in a matrix or gel that is very characteristic of the infected environment (e.g. a wound). This gel like material, known as biofilm, presents a physical barrier to therapeutic agents. Secondly, the approach is rendered less effective in an environment that is lacking in oxygen, precisely the conditions that occur in wounds. In this project we will employ ultrasound together with our novel formulations to (i) disrupt the barrier presented by biofilm so that the drugs can access and kill the bacterial cells, (ii) increase the oxygen content in the infected micro environment to enhance treatment and (iii) deliver the overall therapy deeply into tissues as a result of the ability of ultrasound to pass easily through tissues. A collateral benefit of using ultrasound together with our formulations is that it can be used to increase the blood supply to the wound and this can, in turn, increase the delivery of drugs to those tissues. In overall terms the approach we will use is designed to delivery antimicrobial effects to areas of infection that would otherwise be difficult to access and treat.

What 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 offers the potential to overcome antimicrobial resistance by treating human and animal infections that are currently resistant to conventional therapies.

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

The project will employ approximately 1000 mice over a 2 year 2 months 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?

Under general anaesthesia animals will have an abrasion wound made on their back by scraping the surface of the skin. A solution of bacteria will be put onto the abrasion and then substances will either be put onto the wound or injected. Ultrasound of light will be applied to the wound. The wound is likely to be painful and anesthesia, analgesia, together with careful monitoring, will be employed to mitigate against this. In these studies, careful and frequent monitoring will be employed to preclude stress as a result of pain. Death because of anesthesia is possible, although this is unlikely with careful monitoring of dosing and welfare. Animals will be housed singly to preclude cross contamination and interference with the wound. This will be managed by providing additional bedding/nesting materials and providing additional environment enrichment. Exposure to either the drugs or stimuli pose no risk as these have been shown to be harmless at the doses employed. In all cases, animals will be killed within 48 h of starting the experiments. Any adverse effects are likely to be moderate in severity.

Application of the 3Rs

Replacement

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

Replacement

In all studies, we extensively employ *in vitro* (mammalian cell/microbial cultures) and *ex vivo* (a commercially available human skin model) to test our formulations for efficacy and toxicity prior to embarking on animal studies. However, none of these models can recapitulate the unique architectural and physiological environment presented by an infected wound *in vivo*. Non-protected animal alternatives such as fish fry do not have the same skin architecture as mammalian skin.

Reduction

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

Reduction

As a result of a wealth of data already generated in studies using *in vitro* and *ex vivo* models, some studies *in vivo*, we can employ those data to calculate the minimum number of animals required to provide statistically robust data from experiments. This, together with using the most advanced *ex vivo* models currently available to fully characterize our formulations prior to embarking on animal studies minimizes our dependence on *in vivo* studies and ensures that only the most promising formulations are taken through to *in vivo* studies. Although dependent on the formulation to be employed we typically employ groups that consist of 6 animals per group. Since the effects we wish to observe result from a combination of the stimulus and the formulation, it will be necessary to employ treatment groups that experience no treatment, treatment with the stimulus alone and treatment with the formulation alone as controls. In our studies, animals will be randomly distributed to each group and blinding will occur at the *post mortem* tissue analysis stage. Data from each animal will be maximized by *post mortem* harvesting of relevant tissues (wound) and internal organs for subsequent analysis. These will be stored for additional analysis and for distribution to others working in the field.

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

An abrasion wound infection model will be used, primarily because it is an internationally accepted model of wound infection, it provides a wound with compromised vasculature, so we can test our delivery system, it is amenable for use over a short time frame and provides an appropriate model wound architecture for assessment of interventions to treat infections that result in biofilm. Lower sentient animal models such as fish fry present a different wound architecture, vascular system and immune response configuration that would be insufficient to test the aspects we wish to explore in these studies. To provide effective pain control, Tramadol and paracetamol will be administered in drinking water throughout the protocol and this will start at least two days prior to wound generation to ensure efficient pain management. If determined through careful monitoring throughout, this will be supplemented with additional use of local anesthesia/analgesia to control pain. All pain control measures will be agreed in advance with the NVS (named veterinary surgeon). In addition to employing anesthesia and analgesia during our experiments, animals will be closely monitored using recognized metrics such as the grimace scale, body condition scoring, monitoring weight, respiration, response when disturbed and body temperature to preclude animal discomfort. Monitoring body temperature by non-invasive infra-red thermography will minimize stress from repeated handling. In these studies, animals will be singly housed to preclude cross contamination and interference with the infected wound. Any resulting stress will be minimised by providing additional nesting materials and environmental enrichment. Any animal displaying discomfort, as determined by specific monitoring metrics, will be euthanized.

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 Acute Respiratory Distress Syndrome: From Pathophysiology to Therapeutic Intervention
Key Words	ARDS, MSC, MSC extracellular vesicles based therapies
Expected duration of the project	5 years

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):

Acute Respiratory Distress Syndrome (ARDS) is a serious condition that affects about 1 in 5 people in intensive care. Sadly, 30–50% of those affected do not survive, and those who do often have long-term lung problems that can greatly reduce their quality of life. Right now, there are no effective treatments for ARDS, so there is an urgent need for new medicines.
ARDS happens when the lungs become severely inflamed, causing the immune system to go into overdrive. This leads to the build-up of fluid in the lungs, making it hard for people to breathe properly. The mechanisms of the disease pathology are not fully clear yet.
This project aims to study mechanisms of disease onset and progression and to test new potential treatments, including Mesenchymal Stem Cells (MSCs), using mouse models that mimic ARDS in humans. We will closely examine how these treatments affect different types of lung cells, including those involved in the immune response and those that form the lining of the lungs and blood vessels. By developing and testing these models, we hope to gain new insights into how ARDS works and how it might be treated. This research could help guide the development of new therapies to improve the outcomes for patients, reduce suffering, and slow the progress of the 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)?

At the moment, there are no effective medicines to treat Acute Respiratory Distress Syndrome (ARDS). This project aims to help change that. The goal is to gather the knowledge needed to develop new treatments that could one day be tested in early clinical trials with patients.
In the long term, it is hoped that this research will lead to new ways to ease the suffering of people affected by this serious and often life-threatening lung condition.

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

Mouse
Approximately 4000 over a 5 year period (estimate based on continuous use of mice between the 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?

Inflammation is normally the body's natural way of healing and fighting infection. However, in certain conditions like Acute Respiratory Distress Syndrome (ARDS), this process can become uncontrolled and cause serious damage to the lungs, making it difficult or even impossible to breathe.
To better understand ARDS and how it affects the lungs, researchers will use mice to model the condition. In these studies, pneumonia will be triggered by giving the mice certain bacteria or bacterial substances, which causes lung inflammation similar to what happens in people with ARDS. This may lead to faster breathing, less movement, or changes like a hunched posture or ruffled fur. These symptoms are usually mild and temporary, and the animals will be closely watched and cared for throughout.
Most of the studies will be carried out using normal mice, but in some cases, genetically modified mice will be used to help researchers understand how ARDS develops or how new treatments might work. These genetic changes are not expected to affect the animals' well-being.
Majority of the procedures planned will range from mild to moderate in terms of discomfort. Mice showing signs of illness will receive supportive care, such as extra fluids, oxygen, or a high-energy diet. If a mouse does not recover within 6 hours, it will be humanely put to sleep to prevent any ongoing suffering. All animals will be humanely euthanised if they reach a point where recovery is no longer likely, in line with pre-set humane endpoints.
Severe protocols are included in this project to assess lung injury caused by live bacterial infections which is the common cause of ARDS in human patients. It is expected that more animals in these protocols might develop non-resolving lung injury and symptoms of respiratory distress. Animals will be closely monitored and treated as appropriate with supplemental oxygen, subcutaneous fluids and/or high energy diet and if the symptoms will not resolve within 6 hours then 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

This project will be carried out alongside work with primary human cells, ex vivo human lung tissue explant culture and patient samples. Whilst these approaches can be used in addition to animal work, they cannot replace it. This is because neither cell lines nor patient samples can replicate the complex inflammatory environment needed to study the immune system as a whole, and it is not possible to alter specific immune cell types in human volunteers. Therefore, we have no alternative to using animal models at this time.

Reduction

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

Reduction

This project will utilise state of the art technology where available, i.e. in vivo imaging and in doing so this will substantially reduce the numbers of animals required for experiments because the animals will not need to be sacrificed until the experimental endpoint as opposed to 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

Mice are the most suitable species for this project for a number of reasons, including the availability of suitable mouse models of lung disease to work with, the ability to breed mice quickly and the many experimental techniques and equipment which have been designed specifically for use with mice.
All studies will be designed to prioritise animal welfare while being suitable for the purpose of the experiment.
The protocols included in this project have been carefully considered to minimise harm to the animals. Throughout the course of this project, there will be regular discussion with animal care and welfare officers, vets and other animal unit staff in order to make sure everything possible to improve animal welfare is being done.

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Circadian Clock Disruption in Diabetic Retinopathy
Key Words	diabetes, diabetic eye disease, circadian rhythms, circadian disruption
Expected duration of the project	5 years

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):

Diabetic retinopathy (DR) is one of the most common and serious complications of diabetes. It damages the retina, the light-sensitive part of the eye, and is a leading cause of vision loss around the world. Despite regular screening and advances in treatment, there are still no approved therapies to stop or slow DR in its early stages, when intervention would be most effective.

In recent years, we have learned that DR is not just a disease of the blood vessels in the eye, but it also involves the nerves and immune cells of the retina. Importantly, these changes often start *before* any visible damage or symptoms appear. That's why scientists are now turning their attention to the earliest events that trigger this disease.

One of the most exciting new areas of research involves the circadian system, our internal biological clock that keeps the body in sync with the 24-hour day and night cycle. This clock helps regulate sleep, metabolism, hormone levels, and even immune responses. It turns out that clocks are also found in the eye and regulate our visual precision. In diabetes, these daily rhythms are often disrupted. Disrupting the eye's natural rhythm may make the retina more vulnerable to damage.

Our research aims to understand exactly how diabetes affects the body's internal clock, especially in the eye, and how this disruption might accelerate diabetic eye disease. We're also exploring ways to restore this rhythm to prevent or slow down vision loss.

Our Project Goals:
Aim 1: Understand how diabetes affects the circadian system in the eye and beyond
We want to find out:

- When and how the eye's clock starts to break down in diabetes.
- Whether different parts of the eye's clock are affected.
- If changes in blood sugar levels interfere with the clock's function.
- If eye disease in diabetes affects how well the circadian system responds to daily light and dark cues.

Aim 2: Discover how a broken clock worsens diabetic eye disease.
We will study:

- Whether disrupting the normal day-night rhythm (like shift work or irregular sleep) makes diabetes and diabetic eye disease worse.
- How specific parts of the eye's clock contribute to the damage seen in diabetes.

Aim 3: Test ways to protect the eye by fixing the clock.
We will try:

- New approaches that time the treatments to when the body is most responsive (called **chronotherapy**).
- Drugs that directly reset the body's clock (**chronomodulators**).
- Lifestyle changes like time-restricted feeding, controlled light exposure, and daily routines to support a healthy rhythm in the eye.

What 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 aligns with a key goal set by the UK (United Kingdom) research foundation Diabetes UK, steering Group on Complications: *"For people who already have diabetes, we must find new ways to stop devastating complications that damage health and quality of life in the long term."*

If we can prove that a disrupted body clock contributes to diabetic eye disease and more importantly, show that we can fix this, we may open the door to completely new ways of preventing blindness in people with diabetes. Because the circadian system is highly responsive to both behaviour and medication, this work has strong potential to lead to simple, practical, and effective strategies that protect vision and improve quality of life.

In the short-term, we will gain a better scientific understanding of how the circadian clock influences the eye's immune and vascular systems. We will identify clock-targeting drugs that could be developed into treatments for retinal diseases and we will put strong foundation for future research, with the most promising therapies.

In the long term, this research might provide new and effective therapies for early interventions before signs of diabetic retinopathy arise. Because we are also looking on how the body's rhythms influence the disease, we will provide the foundations for personalized treatments where they are timed with the body's natural rhythms for maximum effect. Finally, this research will provide evidence for new prevention strategies to slow down the disease including sleeping regulation, meal timing, light therapy and medications to reset the body clock.

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

We plan to use mice which display symptoms of diabetes. We will use pups, adult and aged mice, which display symptoms of diabetes and retina degeneration. We will also use genetically altered animals that allow the visualisation and tracking of different cell types in the retina.

The research project will last 5 years and we estimate to use approximately 4000 animals during that 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?

In this project we will breed or make mice diabetic and will keep them until they develop diabetic eye disease. Both wild type and genetically modified mice will be used in this project. The genetically modified mice will not have a key protein in their eyes that keeps the eye's natural circadian rhythm.

In some groups, we will have the mice experience circadian disruption by manipulating the light cycle, to cause jet lag, similarly to what people experience when they travel across time zones. Because to develop diabetic retinopathy, we need to keep mice for almost eight to nine months, we introduced three different acute models of eye damage: one that causes ischemia in the eye and damages the vessels of the eye, one that affects the photoreceptors in the eye, and one that causes uncontrolled vessel proliferation to simulate diabetic proliferative disease, that otherwise diabetic mice do not show, despite being a major effect of diabetes in the eyes in humans. Some of these models are reversible. Using these models, we can induce damage in the eye of the diabetic mice that can simulate better what happens in humans. Our goal is to test the role of the circadian clock of individual cells within the eye in the progression of diabetic eye disease and its impact in overall metabolism of the body.

While a diabetic model by itself causes moderate severity, the prolonged duration of diabetes and the introduction of acute eye damage deems these protocols severe. Common adverse effect from diabetes includes weight loss, susceptibility to infections, impaired wound healing, increased susceptibility to infections, and general distress. Damage to the eyes, can cause temporal blindness, distress and pain in the eyes.

Mice that exhibit severe signs of distress will be culled humanely. At the end of each study, mice will be culled, and tissues will be collected for further histological examination.

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 consists only part of the activities within the group that examine the impact of circadian disruption using in vitro and patient derived cell approaches.

- We use cell cultures and advanced lab models (like co-cultures) to study how different rhythms from different cells in the eye communicate under diabetic conditions.
- We are actively testing retinal organoids—mini three dimensional eye-like structures grown from human cells. While promising, they currently don't contain blood vessels, which are key cells affected in diabetic retinopathy.
- Our team is working to improve in vitro systems, making them more realistic by simulating the diabetic milieu found in the diabetic eyes (high blood sugar, low oxygen, inflammation, toxic lipids etc)
- We also use patient-derived cells to make our findings more relevant to human health.

We only use live animals when there is no alternative, and only for the parts of the project where a full, living system is necessary. Our body's internal clock operates on many levels, through brain signals, local tissue clocks, and feedback from the whole body and only in a whole living organism, like a mouse, we can see how these different layers interact in real time. Moreover, diabetes affects all these systems at once. This kind of multi-layered interaction simply can't be replicated fully in a petri dish.

By combining animal studies with cutting-edge lab techniques, we're doing everything we can to reduce animal use while still advancing science that could transform care and protect vision.

Reduction

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

Reduction

While animal models are sometimes necessary, we use a range of strategies to minimize their use.

We carefully plan our experiments using statistical calculations to ensure we only use the number of animals needed, and we design studies that allow us to collect multiple types of data from the same animal over time.

We use non-invasive techniques like advanced imaging and behavioural monitoring to reduce the need for repeated sampling.

We include both male and female mice in our studies to limit the number of mice needed for breeding and make our results more broadly applicable.

We also use specialized mouse models that let us monitor circadian changes without needing many time-point collections.

Where possible, we replace live animal use with lab-based approaches, such as studying retinal tissue outside the body to examine how different cell types interact over time.

By optimizing the timing of sample collection, we improve data quality and reduce the number of failed experiments.

We also share our data and collaborate with other researchers to avoid duplicating experiments.

All of our work follows the highest ethical and scientific standards to ensure that animals are used only when absolutely necessary and that their use yields the greatest possible value.

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 take great care to minimize animal suffering and follow ethical practices in our experiments. Our protocols are classified as severe severity, however, most animals experience moderate severity and we aim to reduce harm through several refinements. For example, we use the lowest effective dose of streptozotocin, a toxin used to induce diabetes, house the mice in a heated environment, and provide insulin as needed. We reduce the number of anaesthesia sessions by performing multiple tests under one session and protect the mice's eyes with artificial tears. We use both genetic and induced diabetes models, choosing the streptozotocin model when possible, to reduce the number of animals needed for breeding, but also using the genetic models when appropriate, as it causes less severe disease. Finally, when euthanizing animals, we use humane methods to minimize their suffering but also to preserve organ structures for analysis, helping us avoid repeating experiments.

NON-TECHNICAL SUMMARY (NTS)

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Word limit; 1000 words

Project Title	Investigation of a Photosensitiser Peptide Conjugate for Photodynamic Therapy as an Anticancer Agent
Key Words	Melanoma, photodynamic therapy, light, tumour reduction
Expected duration of the project	5 years

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 aims to test and develop a new light activated cancer treatment which operates using photodynamic therapy (PDT). It uses a light-activated compound to kill cancer cells more effectively and safely than chemotherapy. The research will use mice to explore how well this treatment works on melanoma (a serious type of skin cancer), especially in cases where the cancer has spread. The project will look at four main things: whether the treatment can affect untreated tumours elsewhere in the body (for metastasised cancer), whether packaging the drug in nanoparticles improves its effect, whether it works better when combined with existing treatments, and whether it can treat other types of cancer beyond skin cancer, specifically both prostate and breast cancer in the first instance. The goal is to collect essential evidence to support future human trials and eventually provide a more affordable and effective treatment for cancer patients. Animal use is necessary here because the body's immune and tumour responses can't be fully understood in the lab.

What 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 could lead to the development of a safer, more effective cancer treatment for patients with melanoma and possibly other cancers. The new therapy uses normal white light to activate a special compound that targets and kills cancer cells while causing fewer side effects than traditional treatments. By improving how the treatment is delivered and combining it with existing drugs, the research may help treat advanced cancers that are currently hard to manage. If successful, this work could lead to better survival rates, fewer treatment side effects, and lower healthcare costs. It could also provide new insights into how the body's immune system fights cancer, helping researchers design even better therapies in the future. Ultimately, this project aims to bring a promising lab discovery closer to being available as a real-world treatment for patients.

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, the researchers expect to use around **1,400 mice**. These will include different types of mice depending on the specific part of the study, including some that have weakened immune systems or are specially bred for cancer research. Most of the mice will be used to test the main treatment being developed (about 1,000) for three types of cancer; skin, prostate and breast, while others will be used to test how it works alongside existing cancer treatments like immunotherapy or chemotherapy (around 400 in total). All the mice will be carefully monitored, and their wellbeing will be a top priority throughout 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 of the work involves administering cancer cells and testing new light-activated treatments. The main effects expected are those related to tumour growth and to the treatments themselves. All procedures are carried out under general anaesthesia, with pain relief and close monitoring to minimise distress. The mice will be closely monitored throughout all procedures to ensure any discomfort is kept to a minimum. Animals will be housed in accordance with the code of practice with male animals in single sex cages and female animals cohoused, all receiving fresh water and food always available with regular cleaning.
Procedures and routes:
Tumour implantation: Cancer cells will be injected under the skin (subcutaneously), usually on the flank, using a fine needle. This may cause a small swelling that slowly enlarges as the tumour grows. Occasionally, tumour cells will be injected directly into the prostate or breast pads, this involves a small incision to inject the tumour cells directly into the specified tissue, with some sutures to close the wound. All surgery will be carried out using sterile equipment under anaesthesia. Drug administration: Treatments will be given either by intravenous injection (into a tail vein) or intratumoral (directly into the tumour) or intraperitoneal injection (into the abdomen), depending on the compound. These routes may cause momentary discomfort and mild local irritation but no lasting harm. Imaging: Animals will undergo in-vivo imaging systems (IVIS) and ultrasound imaging (US) under brief anaesthesia to track tumour size and organ function. These are non-invasive procedures, and animals recover fully within minutes. Light activation: The treatment area will be exposed to a focused light source under anaesthesia. This may cause mild local heating, redness, or temporary inflammation at the treatment site.

Expected adverse effects may include:
-Small local swelling at the tumour implantation site as the tumour develops.
-Redness, warmth, or mild irritation at the tumour or treatment site.
-Temporary drowsiness or reduced activity following anaesthesia for imaging or treatment.
-Short-term inflammation or minor tissue damage after light activation or drug exposure.
-Mild weight loss or reduced grooming as tumours grow.
-Rare risks from injections, such as brief discomfort or minor bleeding at the site.

Animals will be killed humanely before tumours become large or cause significant discomfort. Anaesthesia and analgesia will be used whenever appropriate. Overall, the expected level of severity is moderate, since any discomfort is short-lived and controlled through careful monitoring and early humane endpoints. No severe or prolonged pain is anticipated.
All mice will be closely checked every day. If any animal shows signs of suffering, it will be humanely put to sleep. At the end of each study, mice will be euthanised under approved guidelines so their tissues can be studied. This ensures we get as much useful information as possible while keeping animal 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

We need to use animals because this project studies how a new cancer treatment works in the whole body, especially how it interacts with the immune system and spreads to other parts of the body. Prior to this proposed work, we have carried out toxicology studies, singlet oxygen quantification, chemical analysis studies and various in vitro cell studies. This has allowed us to determine the mechanism of action and establish dosing regimens and quantities, however, there is no suitable alternative for oxygen supply, immune reaction, and tumour response. Because of this, animal use is unavoidable at this stage. These complex processes can't be fully understood using lab-grown cells or computer models. While we do use non-animal methods like cell studies to guide our work, they can't show how the treatment affects living tissues, blood flow, or immune responses. We have tried to find alternatives by reviewing the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) guidance, Home Office resources, and scientific literature databases (such as PubMed and web of science) to identify validated non-animal alternatives. At present, there are no non-animal systems that can reproduce the interaction between a functioning immune system, a vascularised tumour, and the body's oxygen supply—all of which are essential for photodynamic therapy to work. Using mice helps us gather vital safety and effectiveness data before moving on to human trials.

Reduction

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

Reduction

We will keep animal numbers as low as possible by carefully planning each experiment to get the most useful information from the fewest animals. Before any work begins, we will use statistical power calculations and guidance from the NC3Rs Experimental Design Assistant to determine the minimum number of animals required for reliable results. We will also run small pilot studies first, re-use data where possible, and make sure each mouse provides as much information as it safely can—for example, imaging, regular monitoring during the experiment with, and tissue and organ analysis after the study ends. This helps avoid wasting animals and ensures their use is truly 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

We chose mice because they are the smallest and most widely understood mammalian model in cancer research with their biology well documented. They are widely accepted as a suitable pre clinical model for regulatory agencies prior to clinical studies (including the Medicines and Healthcare products Regulatory Agency (MHRA)) . They allow us to study how the treatment works in a living body, including how the immune system and tumours respond—things we can’t test fully in the lab. The mouse models we use are carefully chosen to match the type of cancer being studied.

To reduce harm, we use the least invasive methods possible, provide pain relief when needed, and closely monitor the animals' health. If any mouse shows signs of suffering, it will be humanely put to sleep to avoid distress.

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 pre-clinical analysis of novel therapeutic agents in the treatment of inflammatory diseases
Key Words	Rheumatoid arthritis, inflammatory bowel disease, pancreatitis, mouse models, novel drugs, therapy
Expected duration of the project	5 years

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 overall aim of this program of work is to provide a fee-for-service to clients (such as academic researchers, small biotech companies and large pharmaceutical companies) whereby we use animal models to assist them in the discovery and development of new therapies for the treatment of inflammatory disease (such as inflammatory bowel disease or rheumatoid arthritis).

In order for us to do this we must do the following: -

1. Find safe dose levels for these new treatments and check if they cause any unwanted effects in specific organs
2. Make sure the treatment reaches the right part of the body and is working as intended. We'll also look for signs in the body (called biomarkers) that could help indicate if the treatment is working in future patients
3. Test how well these treatments work in different models of diseases that are similar to what people experience, to see if they could be useful in real-life medical situations.

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

It is estimated that globally, hundreds of millions of people live with some form of chronic inflammatory disease. There are dozens of types of inflammatory diseases, which can affect nearly every organ system in the body.

Despite the recent advancements in treatment of inflammatory diseases, there are still many types for which targeted therapies are not available. In other cases, therapies are available, but treatment is not effective for all patients and many patients will not respond.

High quality data produced from this project will be used by our clients and when published and/or disseminated, will inform others in the inflammatory disease research community.

If successful, the outputs from the project will form the basis of a pre-clinical package which our clients can utilise to attract clinical collaborators and clinical trial sponsors to translate our work into clinical practice. We have already achieved this aim with previous projects under our previous licenses. We hope that ultimately, benefits will be gained to patients and health care providers and funders. We hope to assist in the discovery of more effective inflammatory disease treatments.

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

Over the 5 years of this license, we expect to use 1,000 mice or rats per year for objective 1, 2,000 mice or rats per year for objective 2, and 3,000 mice or rats per year for objective 3.

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 animals used in our studies have been bred to have a reduced immune system. The animals are kept in high-quality facilities, free from pathogens (disease-causing organisms such as bacteria, viruses, and parasites) and with access to food, water, and environmental enrichment. Animal care staff are highly trained in rodent welfare and ensure animal suffering is minimised. Animals are housed in groups except in exceptional circumstances, for example when aggressive behaviour puts the welfare of the animal at risk or when cage mates have been removed for experimental reasons.

We will use both induced and non-induced animals for our projects. Inflammation is induced by administration of a pro-inflammatory agent systemically e.g. in the drinking water for the DSS (Dextran Sulphate Sodium) induced colitis model, or directly into the organ/tissue of interest e.g. the brain for the intracranial inflammation model. The development of inflammation in the animal after induction may be monitored by physical measurement of an associated side effect e.g. stool consistency in models of colitis, and paw swelling in models of arthritis.

Animals who have inflammatory disease may become unwell; however, these experiments will be stopped before the inflammation 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 inflammatory models will be or have already been trialed in a small number of animals (pilot group) 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 experimental anti-inflammatory drugs are commonly administered orally, injected into the abdomen, injected into the tail vein, or injected under the skin. Occasionally drugs may be administered by injection into the leg muscle, injection into the skin, or via a small pump device that is surgically implanted under the skin. At times these substances may only need to be administered once, but more often they are administered according to a schedule that requires multiple administrations. In some cases, the injection of cells or drugs into the animal may cause transient discomfort but, again, by using only well trained and experienced people, together with analgesics when appropriate, we will minimise this adverse effect and the volumes and routes of administration are chosen to cause the least possible discomfort.
Treatment of animals with anti-inflammatory therapies may also lead to unwanted effects like those experienced by human patients. While humans may experience fatigue or fever soon after receiving the therapy, we observe similar responses in rodents such as reduced movement, hunched posture, and bristling of the fur. Most of these effects will be mild and of short duration; however, some animals may experience moderate effects.

Application of the 3Rs

Replacement

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

Replacement

Although we do many experiments in the laboratory using cells and molecular biology, it is still necessary to use some animals for research so that we can more accurately assess the interaction of therapies with cells and organs within the body. Isolated cells and organs do not reproduce the complex nature of in vivo (in a live animal) biology. The use of animals also allows us to understand inflammation in the organ/tissue of origin. Another important aspect is to understand how the immune system can be harnessed to treat inflammation, and it is not possible to fully recreate these complex interactions outside of a living animal.

We have undertaken a comprehensive literature search using online databases from around the world as well as patent search databases and have considered relevant websites such as NC3R (National Centre for the Replacement, Refinement, and Reduction of Animals in Research) and NC3Rs Oncology Network and FRAME (Fund for the Replacement of Animals in Medical Experiments), to identify alternatives to the use of animals in this specific research project. While there are some models under development that may be ready and useful in the future it is too early in their development to consider their use instead of animals.

We regularly use a range of in vitro methods. These experiments take place in a test tube or laboratory dish, outside a living animal. Such methods may include experiments containing a single cell type or multiple cell types grown together, which allows both direct interaction and indirect communication between cells. These types of studies are well-established, 2-dimensional experiments that are useful to understand the specific ways that our experimental drugs affect cellular function.

Cell-based methods are useful to gain an understanding of the way that our experimental drugs impact the function of different cell types outside the body, but do not adequately test whether the drugs remain stable after they enter the body, can reach the site of the inflammation, or whether they are capable of inhibiting inflammation in a live animal.

None of the alternatives investigated can demonstrate how our experimental therapies are broken down by the body, nor can they provide any information about how to schedule dose regimens. Unlike experiments using animals, they also do not permit identification of specific signals produced in the body which can assist in determining treatment effectiveness in patients.

Reduction

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

Reduction

Studies will be meticulously planned and adequately powered to ensure that sufficient numbers of animals are included to uphold statistical significance, thus avoiding the need for unnecessary repetition. This will be facilitated by our extensive experience with the inflammatory models described in this project, which has allowed us to gauge the minimum number of animals that are typically required to detect statistically significant differences.

Further advice on study design and analysis will also be sought from the literature, from resources such as the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines and the NC3Rs experimental design assistant tool, or from consultations with statisticians where required who can advise on sample size/power calculations and appropriate statistical 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

This project will implement a range of refinement strategies to minimise animal suffering and improve welfare throughout the study. These include: -

- Selection of appropriate models: Animal models will be chosen based on their relevance to human disease and their ability to provide meaningful data with minimal distress. Where possible, models with reduced severity will be prioritised. Models using less sentient species do not share the same similarities seen between that of the rodent and human in terms of complexity of biological systems such as immune systems, organ activities, metabolism, circulatory systems etc. making it harder to study the human response with respect to drug or nucleic acid delivery with those types of models. Currently the zebrafish model is the only other less sentient non embryonic model available that could be utilised for these types of studies; however, the data from this model is still not comparable to the data that is achievable using rodent models at this current time.
- Monitoring and humane endpoints: Animals will be closely monitored using validated welfare assessment tools. Humane endpoints will be clearly defined and applied to prevent unnecessary suffering, including early termination of procedures if adverse effects are observed.
- Minimally invasive techniques: Procedures will be designed to reduce invasiveness, using refined dosing and sampling methods (e.g., microsampling, non-invasive imaging) to limit pain and distress.
- Analgesia and supportive care: Where procedures may cause discomfort, appropriate analgesics and supportive care will be provided. Staff will be trained to recognise signs of pain and intervene promptly.
- Environmental enrichment and housing: Animals will be housed in enriched environments that support natural behaviours and reduce stress. Social housing will be used wherever compatible with the study design.
- Staff training and competency: All personnel involved will be trained in refinement principles, animal handling, and welfare monitoring to ensure high standards of care and ethical conduct.

These measures will ensure that the scientific objectives are met while maintaining the highest standards of animal welfare.

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	Antigen presentation during immune challenge, autoimmunity, and malignancy
Key Words	Immunology, antibodies, autoimmune disease, blood cancer
Expected duration of the project	5 years

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 aims to understand how a type of immune cell called a B cell helps the body recognise and respond to disease. B cells detect pieces of harmful or abnormal material, known as antigens, and show them to other parts of the immune system to trigger a response. We want to learn more about how this process is controlled inside the cell and what happens when it goes wrong.

Using modern genetic tools, we will switch off certain genes in B cells to see how this affects the way they process and display antigens. We'll study these changes both in cells grown in the lab and in carefully designed mouse models.

By doing this, we hope to find out which molecules are important for normal immune function and how they might behave differently in conditions such as autoimmune diseases or blood cancers. We will also explore whether adjusting these processes could help reduce symptoms or slow disease progression.

What 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 help us understand how B cells, a type of white blood cell that plays a key role in the immune system, recognise and respond to infections. By studying how these cells take in and process foreign substances, we can uncover what controls their activity and why this sometimes goes wrong in disease. Ultimately, this work will help us understand the immune system in greater detail and could lead to new ways of treating diseases where the immune system is overactive or out of balance.
These findings could help researchers design more effective vaccines and improve treatments for infections, autoimmune diseases such as lupus, and some types of blood cancer.
As the project progresses, it will also generate important new knowledge about the genes and proteins that control immune responses, as well as the tiny particles cells use to communicate with one another. In addition, the work will help refine laboratory techniques used to study the immune system, supporting scientists around the world in developing better tools and approaches for future research.

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

We will use mice; breeding approx. 2000 animals, and using approx. 4000 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?

Most of the animals used in this project are expected to show only mild and short-term effects.

The substances and genetic tools we use to label cells or alter gene activity are not expected to cause noticeable side effects. Transferring immune cells between healthy, genetically similar mice is also well tolerated. In some cases, if immune cells that can cause autoimmune reactions or lymphoma are transferred, a small number of animals may develop signs of these conditions, which may include skin problems, swelling, ruffled fur, hunched posture, reduced appetite or activity, weight loss, or diarrhoea. Mice will be checked regularly for these signs but they are rare – most mice are expected to remain outwardly healthy, with early disease detected by tests in the lab, for example urine tests, long before clinical symptoms develop. This allows us to act promptly to protect animal welfare.

In some studies, mice undergo a procedure called irradiation followed by stem cell reconstitution. This is done to temporarily reset the immune system so that we can study how new immune cells develop and function. Irradiation involves exposing the mouse to a carefully controlled dose of radiation. The procedure itself is short and painless, and the mouse does not feel the radiation as it is given. However, over the following days, the radiation reduces the number of blood and immune cells in the body. During this time, mice may feel more tired than usual and are more vulnerable to infections.
To rebuild the immune system, healthy stem cells, which are the cells that can develop into all types of blood and immune cells, are then given to the mouse, through an injection. The stem cells travel to the bone marrow, where they begin to produce new immune cells over the next few weeks. Throughout this process, mice are monitored closely. They are housed in specially protected cages to reduce the risk of infection and may receive antibiotics and supportive care. Most animals recover well as their immune system is restored.

For animals that receive an immune challenge (for example, an injection to trigger an immune response), different injection sites will be used each time whenever possible. The location of every injection will be carefully recorded so the same area is not used again. If any mild skin irritation or small lesions occur, these will be treated with suitable topical cream after consulting the Named Veterinary Surgeon or Animal Welfare Officer. Only the smallest possible dose of any microbial agent will be used, as the goal of this work is to study how the immune system recognises and responds to antigens, not to cause illness or long-term infection. All animals are expected to recover fully and will be humanely killed at the end of the experiment. In some studies, newborn mouse pups are exposed to a mild or carefully controlled dose of influenza virus to help us understand how the immune system responds to infection early in life. The pups are monitored closely for any signs of illness, such as temperature and reduced activity, and the doses used are chosen to cause only temporary, mild symptoms with full recovery expected.

Application of the 3Rs

Replacement

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

Replacement

Our research mostly uses human B cells grown in the lab. These cell lines allow us to study basic cell biology, such as how cells grow, send signals, and interact with other proteins. These cell models can be maintained for a long time, provide a large amount of material and have human relevance; they have allowed us to study the function of several cellular proteins and comprise a major part of the data in our publications.
However, it is not currently possible to fully recreate the complexity of the immune system in a dish. Studying how B cells develop, or how mature B cells respond to infections, requires the full network of cells, tissues, and chemical signals found in a living body. While new advances like human organoids (mini-organs grown in the lab) are promising, they cannot yet mimic the dynamic environment of a real immune response. We study these developments closely and test non-animal alternatives where possible.
Finally, some aspects of the immune system, like differences between males and females, or the effects of inflammation in autoimmune diseases, can only be properly studied in living animals. Therefore, carefully monitored mouse experiments are necessary to answer our research questions. Mice are the most suitable animals for this research because they are the smallest mammals that closely resemble humans in how their bodies and immune systems work.

Reduction

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

Reduction

We have a large animal facility that allows us to share mouse strains between research groups. This helps reduce the total number of animals needed.
In our experiments, a single mouse can often be used for multiple types of analysis, such as different cell and antibody measurements, which also reduces the number of animals required. We use both male and female mice to make sure our results are applicable to both sexes.
Mice are randomly assigned to different experimental groups to avoid bias, and experiments are designed so that measurements are taken “blind”, meaning the researcher does not know which group each mouse is in while collecting data. We also account for factors like cage placement to make sure the results are accurate. These careful design steps help us detect meaningful differences using the smallest number of mice possible.
From previous studies, we have estimates of how much variation to expect in measurements, which allows us to calculate the minimum number of animals needed for each experiment. For example, in some experiments, we can achieve reliable results with as few as nine mice per group. For each new experiment, we calculate the number of mice required based on scientific publications and small pilot experiments, ensuring we always use the smallest number of animals 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

The mouse models and genetic techniques we use are well established and effective for studying how B cells take up and process antigens. There are many tools and methods available for working with mice, which allows us to design experiments carefully using the most refined procedures. For example, we have improved methods for temporarily removing stem cells and reintroducing them, which causes less stress and fewer side effects than previous methods.
Most procedures in this project are expected to have only moderate effects on the animals. However, some genetic changes are unpredictable, and a small number of animals may be more vulnerable to infections or show reduced immune responses. To protect them, any animals with reduced immunity are housed in individually ventilated cages and given preventative antibiotics. We also use younger mice where possible, as they tend to recover more quickly.
All procedures, such as injections or cell transfers, follow established best practices to keep animals safe and comfortable. For models of infection, we use very low doses that result in minor signs of disease and allow full recovery.
All staff involved are trained in animal care, research techniques, and experimental design. Skilled handling ensures that experiments are performed quickly and accurately, which reduces stress and discomfort for the animals and improves the reliability of the results.
We also use several approaches to further improve welfare, including clear humane endpoints, careful monitoring before and after experiments, and team discussions to refine procedures. In models prone to autoimmunity, we use urine tests to detect early cellular changes, before significant clinical symptoms. By identifying disease at this early stage, we can intervene promptly or apply humane endpoints, helping to minimise discomfort and prevent more severe illness in the mice.

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	Hormonal and Metabolic Studies
Key Words	Diabetes, obesity, peptide, therapeutics, metabolism
Expected duration of the project	5 years

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

Purpose	
Yes/No	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes/No	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes/No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes/No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes/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/No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
Yes/No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
Yes/No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
Yes/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 Type 2 diabetes are major, growing health emergencies, and many current treatments are not effective enough or have significant side effects. This project aims to discover and test a new generation of more effective treatments.

Our research is primarily focused on developing new medicines based on natural signals (hormones) that our gut and bodies produce. These natural signals are very powerful: they tell the brain when we are full, and they help the pancreas release the right amount of insulin to control blood sugar.

Our key objectives are:

1. **To design better medicines:** We will create and test 'multi-action' compounds that can perform several jobs at once such as reducing appetite, improving blood sugar, and protecting the pancreas all in a single treatment. We will also search for brand-new compounds from natural sources (like plants or animal peptides – venoms makes them sound toxic to human – do you want to use peptides instead?) that could work in new ways.
2. **To protect the pancreas:** A key goal is to see if these new drugs can protect the vital insulin-producing cells in the pancreas. We want to find out if our treatments can stop these cells from being damaged by diabetes or even help the body to regenerate new ones.
3. **To address related health problems:** We are investigating the strong link between obesity/diabetes and infertility, particularly in conditions like Polycystic Ovary Syndrome (PCOS) and male fertility. You haven't mentioned male infertility in the background, benefits or plan of work (Added as suggested) A major aim is to test whether our new drugs can improve metabolic health *and* help restore normal fertility at the same time.
4. **To explore cell-based therapies:** For patients who have already lost their insulin-producing cells, we are exploring cell-replacement treatments. This involves testing new, safer places in the body where we can transplant healthy, hormone-producing cells.
5. **To understand the "body clock":** We will also run a novel investigation into the body's internal 'clock.' We want to see if the *timing* of when a drug is given (for example, morning versus evening) can make it more effective, which could lead to better treatment schedules for patients.

All new compounds will first be tested in our laboratories using cell models to check for safety and effectiveness. Only the most promising candidates will then be studied in highly regulated rodent models of diabetes and obesity. These essential preclinical studies are the necessary step before any new discovery can be advanced toward clinical trials 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 primary benefits of this project will be a deeper scientific understanding of obesity and diabetes, and the development of new treatment options to move towards clinical trials.

During the 5-year period of this project, we aim to:

1. **Advance scientific knowledge:** We will gain new insights into how obesity and diabetes work, including how they affect other health issues like fertility and the body's natural rhythms.
2. **Discover new treatments:** We hope to identify new, more effective medicines that are better than current options, as well as new ways to use existing drugs.
3. **Help patients:** By developing new drugs and improving cell-based therapies, our long-term goal is to provide better health outcomes for people living with diabetes and obesity, and also for those with related conditions like infertility.

We will share our findings with the scientific community through publications and will protect our discoveries (intellectual property) to help attract partners who can turn them into real-world medicines. Given the essential, early-stage nature of this research, any direct benefit to patients from these specific discoveries is realistically 10 years away or more.

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

We plan to use established mouse and rat models of diabetes. Approximate numbers of rodents to be used in the project is 4500 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?

Adverse effects are expected to be in the mild to moderate range.

- **Disease Symptoms:** Animals will experience the actual symptoms of diabetes or severe obesity. For diabetes (induced either genetically or by a specific chemical that damages insulin-producing cells), this includes excessive thirst, frequent urination, and weight loss. For obesity (induced by high-fat diets), animals will experience massive weight gain, sluggishness, and metabolic strain.
- **Procedural Stress:** Animals will experience temporary stress from being handled, restrained, and given test medicines (either via injection or a soft tube passed into the stomach). To test how their bodies process sugar, they will frequently have their food removed (fasted) for up to 16 hours. Repeatedly taking small blood samples from surface veins will cause minor, temporary discomfort.
- **Housing Changes:** Some animals will be temporarily housed individually in special observation cages (without their normal bedding for up to 48 hours) to measure their metabolism, or placed in altered light/dark cycles to disrupt their body clocks. Both of these cause temporary psychological and physical stress.
- **Surgical Harms:** Some animals will undergo surgery under general anaesthetic to implant small devices under the skin (such as small continuous medicine pumps or supportive structures holding cells). Harms associated with surgery include post-operative pain and a small risk of the wound opening or becoming infected.
- **Unexpected Side Effects:** Because we are testing new medicines, animals might experience unexpected side effects. The most severe risk is a sudden drop in blood sugar to dangerously low levels, which can cause tremors or severe weakness.

Application of the 3Rs

Replacement

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

Replacement

We use an extensive screening process for all compounds at the laboratory bench before progressing to animal studies. Such tests include various experiments in cell lines to determine biological activity and mechanism of action, as well as studies to indicate likely stability and duration of biological action of our compounds. We also test toxicity in cell lines, as well as the ability of compounds to positively affect the growth and survival of insulin-secreting cells in the pancreas. While advanced non-animal technologies like 3D (three-dimensional) organoids and 'organs-on-a-chip' are incredibly useful for studying individual tissues which we do intend to use, they cannot yet replicate the complex, whole-body interactions between the brain, gut, liver, and pancreas that drive systemic diseases like diabetes and obesity.

We also employ acute *in vivo* studies before progressing towards longer-term sustained drug administration regimens in animals. Only compounds deemed suitable will be progressed to *in vivo* studies.

Unfortunately, *in vitro* systems are unable to accurately mimic the *in vivo* environment, and thus animal studies are a necessity in drug development. In addition, no single animal model can accurately mimic the human form of obesity-diabetes, thus we need to employ various models of different diabetes aetiologies.

We have also found that the metabolic benefits of novel treatments often occur through distinct and sometimes unexpected mechanisms, relating to redundancy/plasticity in physiologically relevant biochemical pathways, such important mechanisms and insights could not be established with *in vitro* testing.

Data derived from animal models of disease is still a cornerstone in the successful development of new drugs. However, to ensure we implement new non-animal technologies as soon as they become available, we will continuously monitor the horizon by regularly reviewing literature from the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and attending relevant scientific conferences.

Reduction

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

Reduction

We always make use of statistical power calculations to determine the appropriate number of animals required to achieve likely significance in relation to our primary endpoint. As such, free software available at http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html is routinely used for our animal studies. We also have extensive experience in designing and reporting on *in vivo* studies and this knowledge will also be used to assure minimum numbers of animals are used. For randomisation of groups, animals are given a cage number and then randomly assigned to groups based on matching of body weight and blood glucose levels. Whilst blinding of the researcher is not always possible because of drug preparation requirements, this is practiced where possible, however, the measurement of experimental outcomes is strictly blinded.

Moreover, analyses of sample e.g. immunohistochemistry, is conducted in a blinded manner using for example colour or numerical coding by a colleague, with treatment allocation only revealed to the researcher after analyses are completed. To prevent the wastage of animals, we employ highly efficient breeding strategies for our genetically altered colonies. We carefully match our breeding programs to exactly meet our experimental needs, continuously monitoring colony sizes using database tracking. This strict colony management ensures we do not over-breed or produce surplus animals that cannot be used in our 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

Obese diabetic (*ob/ob*) mouse, diabetic (*db/db*) mouse and Zucker fatty (*fa/fa*) rat all display a defect in their leptin or leptin receptor which leads to the development of diabetes-obesity syndrome which resembles the human Type 2 diabetic condition. More akin to the human situation, we will also make use of diet-induced not environment but diet forms of obesity-diabetes such as prolonged high fat or cafeteria feeding. To replicate human lifestyle we implement cafeteria feeding of highly palatable, high-fat, sweet diet, consisting of a variety of highly palatable foods (such as bacon, cheesecake, chocolate, peanut butter, however, we mainly use diet-induced models by feeding animals commercially formulated, standardised high-fat or high-carbohydrate diets alongside standard rodent diet, to induce overfeeding and obesity.

In terms of progression of drugs to the market, Pharma require proof of drug efficacy in more than one rodent models of obesity-diabetes. As such, these models are the most widely used, well-established and best representative models of disease including genetic, dietary, and chemically-induced models..

All animals will be housed appropriately and cared for by expertly trained personnel. We will endeavour where possible to train animals to cooperate with procedures such as blood sampling to help minimise stress, and provide appropriate housing that allows the expression of species-specific behaviours, such as nesting opportunities for mice.

In addition, use of sophisticated metabolic cages to assess eating behaviour and metabolic rate, as well as X-ray equipment to monitor body composition, allows us to generate more data for a single mouse. Moreover, use of biotelemetry to assess temperature, activity and blood pressure will also allow for increased information to be gained from a single animal. Further, advancements such as use of osmotic pumps for drug delivery and innovative techniques like implanting cells into the eye, which allows us to monitor cell survival non-invasively using a microscope will minimise suffering and improve animal welfare.

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 Telemetry Programme
Key Words	biotelemetry, electronic tags, tagging, tracking, Atlantic salmon, elasmobranch, fish migration, conservation, stock assessment,
Expected duration of the project	60 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;
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):

Many fish species make remarkable long-distance migrations between freshwater rivers and the open ocean. Despite decades of research, scientists still do not fully understand the exact routes these journeys take, where fish are most vulnerable during migration, or how changing seas and human activities are affecting their survival. This programme aims to improve that understanding by attaching small electronic tags to fish and following their movements through rivers, estuaries and the sea.

The project studies fifteen species found in rivers and coastal waters in Northern Ireland and Ireland, including Atlantic salmon, sea trout, European eel, cod, and several species of shark and ray. It will: map migratory routes of each species through river, estuarine and marine environments; identify migration stages where fish are most at risk; gather biological data needed to manage fish stocks sustainably; locate critical habitats; and build predictive models of how climate change and coastal development will affect these species in future.

What 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 produced will directly support the conservation and management of some of the United Kingdom's and Ireland's most threatened fish species, including Atlantic salmon and European eel — both facing serious population declines — and several shark and ray species protected under UK and European law. For commercially fished species such as cod and sea bass, the programme will produce independent biological data improving the accuracy of stock assessments and helping ensure fishing is managed at sustainable levels. All data will be shared publicly through international scientific databases and published in peer-reviewed journals. In the longer term, the predictive models developed will help governments and agencies plan for the impact of climate change and new coastal developments on fish populations.

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

Up to 4,100 wild-caught fish will be used under Protocol 1 over the 60-month licence period, with up to a further 1,200 under Protocol 2, 100 under Protocol 3 and 100 under Protocol 4, giving a programme total of up to 5,500 animals across all protocols and species. The largest groups under Protocol 1 are Atlantic salmon (up to 1,000) and sea trout (up to 1,000). Larger, rarer species such as Atlantic bluefin tuna, basking shark and the various shark and ray species are limited to a maximum of 100 individuals each per protocol over the entire licence 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?

Fish are caught from the wild using standard fishing methods appropriate to each species. Before any procedure takes place, every individual is assessed for health and condition. Any individual showing signs of illness or injury is not tagged; depending on its condition, it is either released immediately or, where it is found to be suffering and would not survive release, it is humanely killed without delay. Animals passing the health assessment are then weighed and measured. Tag burden is calculated as the effective weight of the tag relative to the fish's body weight; animals for which no suitable tag falls within the species-specific threshold (typically ≤2% of body weight, with a higher limit of ≤3% for European eel) are released without procedure. Only animals meeting the burden threshold proceed to tagging.

For most species, a small electronic tag is surgically implanted into the body cavity under anaesthetic — a procedure similar in principle to fitting a small device inside the body. This is classified as Moderate severity. The fish then recover in a tank of oxygenated water before being released. The most common effects are temporary stress during handling; serious complications such as infection or tag-related injury occur in fewer than one in twenty animals based on published evidence for this type of procedure.

For larger species where internal surgery is not appropriate or practicable, tags are attached to the outside of the fish using a plate-and-wire saddle system, a titanium dart anchor, or a spring-loaded fin-clamp. Saddle and dart attachment are classified as Moderate severity as they involve attachment hardware that remains with the animal for an extended period; the saddle mount is a permanent attachment following satellite tag release and its welfare implications have been fully assessed on a case-by-case basis. Fin-clamp attachment is classified as Mild severity as it involves no tissue penetration and the tag detaches automatically within two weeks. All animals are released back to the wild after tagging once they have recovered and are swimming normally. Any animal that does not recover adequately is humanely killed without delay. Death is not an intended outcome of any procedure; euthanasia is applied only as a welfare contingency.

Electronic tags can detect movement patterns consistent with predation events in some circumstances, and the absence of further detections may indicate mortality; however the precise cause of death cannot be determined from tag data alone in the majority of cases.

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 study where wild fish go, how fast they travel, or the conditions they experience, without following real animals in real environments. Computer models exist but require primary tracking data for validation and cannot replace actual movement data from free-ranging animals. Electronic tagging of wild fish is the only available method that can provide the required information.

Reduction

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

Reduction

Statistical analysis was used to calculate the minimum number of fish needed to obtain reliable results, with expected losses from natural mortality and tag failure built into the calculations. All data are deposited in international shared databases so that other researchers do not need to repeat the work. Strict annual and per-river limits are set for every species and protocol. Where possible, multiple data types are collected from the same individual during a single capture event, reducing 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

The tagging method used for each species is chosen to cause the least possible harm while generating the data needed, following a defined decision hierarchy. Tag sizes are confirmed within the species-specific burden threshold before any procedure proceeds. Fish are handled for the shortest possible time using wet surfaces, gill irrigation and eye covering where required to reduce stress. Anaesthetic is given for all surgical and wire-attachment procedures. Animals are carefully monitored after tagging and released only when they are swimming normally. Trained personnel able to humanely kill an animal are present at every tagging operation.

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	Badger Intervention in the Northern Ireland (NI) Bovine Tuberculosis (bTB) Regionalisation Project
Key Words	Badger, Ecology, Bovine Tuberculosis, Vaccination, Regionalisation
Expected duration of the project	5 years

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

Purpose	
Yes/~~No~~	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes/~~No~~	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes/~~No~~	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
~~Yes~~/No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

~~Yes~~/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/~~No~~	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
Yes/~~No~~	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
~~Yes~~/No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
~~Yes~~/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 provide data relating to the ecological and disease effects of testing live badgers for bovine tuberculosis (bTB) and removing diseased badgers while vaccinating and releasing test negative badgers (Test and Vaccinate or Remove-TVR). The TVR methodology has been tested previously, however this was in a different area of Northern Ireland (NI) and was trialled in isolation i.e. solely assessing bTB prevalence in badgers without implementing and assessing alongside cattle and people measures and so will add to the growing amount of scientific evidence that is available in this subject area. The hybrid TVR methodology, as described, has not been tested previously. Analysis of this data will be used to update government and industry on policy development moving forward as they attempt to eradicate this 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)?

This project will provide ecological data on badger behaviours and their organisation, as well as their bTB levels, in this area over a five-year period. This data is of intrinsic value as it represents unique information on badgers within an area that has above average incidence of bTB in cattle.
BCG vaccination of badgers has been shown to protect them directly from becoming infected with *M. bovis*. It has also been shown to indirectly protect the cubs of vaccinated badgers.
The effects that this badger intervention approach has on overall bTB herd incidence in conjunction with additional cattle and people measures will inform debate around future bTB policy development.

Scientific data will be collected to inform disease transmission and economic modelling. Quantifying the effect of the badger intervention will allow modelling to be used with more confidence to assist the development of potential future intervention strategies.

Quantifying the costs and field logistics of this badger intervention approach will help inform future policy development.

Whiskers will be taken from dead badgers for investigation of diets by isotope analysis. This research helps improve understanding of badger diet/nutrition, supporting better conservation and management to enhance their health and survival.

Fertility control will benefit badgers as less dense populations are less likely to encounter and transmit infections to nearby badgers/setts.

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

Badgers (*Meles meles*). Based on current badger population estimates and previous cage capture rates, up to 450 unique badgers will be captured per year 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 procedures applied to badgers in this project are: animal capture by cage-trap and cage-side welfare assessment with release of badgers <2kg without any further processing, anaesthesia and detailed welfare assessment, attaching a collar to the animal, obtaining a blood sample, micro-chipping, collection of small hair samples, intra-muscular injection with *Bacillus Calmette–Guérin* (BCG) vaccine or euthanasia if test-positive, and intra-muscular injection with a fertility vaccine if required. Other samples that may be obtained from anaesthetised badgers include hair samples, tracheal aspirates, oropharyngeal swabs, urine sample, rectal swab or enema for faecal samples.

In terms of adverse effects, animals may get stressed, react to anaesthetics, have minor temporary bruising / abrasions and experience momentary discomfort. It is expected that any adverse effects will be temporary and not cause any long-lasting pain or distress. Any injuries will be recorded with cage injuries being specifically recorded. Other injuries will also be recorded separately including any granulomas caused by the fertility vaccine.

Badger cages will be checked as soon as practicable after dawn and all badgers processed as quickly as possible to minimise time caught during daylight hours.
All captured badgers will be health and welfare assessed by a veterinary surgeon cage side. Only animals that are well enough and >2kg in bodyweight will undergo any procedures. Those that are smaller or considered not suitable will be immediately released.

Location collars will be fitted to up to 10 badgers within a sub-area of the whole study area. With collaring there is a risk of long-term skin wounding. This will be mitigated by using collars with a drop-off mechanism and only badgers heavier than 8 kg and a cranial circumference of at least 1 cm more than the neck circumference, will be deemed suitable to receive a collar.

As badgers are being anaesthetised, there is potential for overdose or adverse reaction as for any general anaesthetic. This will be mitigated by wide safety margin of the drugs used and the presence of trained and experienced staff using weight related dosage charts and animals will be under the care of a veterinary surgeon. If any animal shows signs of distress (e.g. laboured breathing) then the effects of the anaesthetic can be reversed using an injectable reversing agent. Animals will be monitored continuously throughout induction and while under anaesthesia.

A veterinary surgeon will be available during badger anaesthesia and sampling procedures to observe any reactions and determine any remedial actions.

At the end, badgers that test negative to the bTB test will be released back into the wild at the point of capture immediately after recovery from anaesthesia. Badgers that test positive to the bTB test will be euthanised humanely by overdose of anaesthetic while still under anaesthesia

Application of the 3Rs

Replacement

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

Replacement

It would not be possible to meet the scientific objectives of the project without the use of live badgers in their natural environment. A reduction in the incidence of bTB in badgers can be achieved through the vaccination of live badgers, but the bTB incidence will also be reduced by the selective killing of bTB infected badgers. A reduction in population density is an additional measure in reducing bTB transmission but it cannot be achieved without fertility controls when combined with selective killing.
BCG vaccination of badgers has been shown to protect them directly from becoming infected with *M. bovis* and so is of direct benefit to those in this study area. It has also been shown to indirectly protect the cubs of vaccinated badgers. The project will allow the collection of detailed information which will enable the creation of valid epidemiological models to inform future badger models, cattle and on-farm biosecurity controls. This collated information will guide the development of better policies bespoke for the environmental factors specific to Northern Ireland.

Reduction

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

Reduction

A single study area of 235km² has been outlined in design mandate so badgers will only be captured in this area. Up to 450 badgers per annum are required to provide data for scientific research. Only test-positive badgers will be removed minimising the risk of TB transmission to other species while test-negative, uninfected badgers will be protected through vaccination.

Blood samples will provide enough serum for an archive sample to also be stored (which can be used for evaluation of other TB detection tests if required). These, along with other samples collected may be used to carry out further tests or to test new diagnostic methods as required.
The use of GPS collars on 10 badgers in adjacent social groups will enable assessment of badger-badger connectivity and will provide sufficient data for this purpose.

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 choice of species and study design has been determined by badger involvement in the epidemiology of bTB. The majority of the methods used are those that have been used in other badger studies.

Each sub-area in the region will be subjected to a 3-week trapping cycle which will involve 1 week of survey for badger activity and cage deployment, 1 week of pre-baiting of cages followed by a third week involving four nights of baiting and setting of traps. Peanuts will be used as bait. Trapping will occur from July to late October. Cage traps will be of sufficient size for large badgers, constructed from square 8- gauge mesh and will have no sharp edges. The traps will be triggered by a twine trip line when a badger pulls on a stone to reach bait at the back of the trap. This releases the trap door, which is then held in place by a brace mechanism. Cages set for trapping will be checked within 2 hours of sunrise and tripped upon inspection then are re-set for capture during late afternoon. Cages will be placed in shaded locations as much as possible and traps will not be set if any official weather warning is issued. These sheltered areas will help to shade from the sun during warm periods and, will be in a location close to natural trees or hedges to protect during periods of wind and rain. Recovery boxes are available also for the badgers' post recovery if indicated by the vet assessment.

All captured badgers will be health and welfare assessed by a veterinary surgeon.
Each captured badger will be scanned to record any existing microchip identification number and thereafter checked to see if they had been previously captured during the calendar year. Temporary markings (clip mark and stock marker) will be used to ensure rapid identification if recaptured within short timeframe. If identified as a recapture, its location will be recorded, and the badger will be released if it is observed to be in good health; and sick or injured badgers will undergo more thorough investigation by a vet, which may require the animal to be anaesthetised.

Badgers will have a pre and post anaesthetic assessment by a vet and will be monitored throughout anaesthesia and during recovery. Post recovery as mentioned the badgers will be monitored in the previously positioned sheltered cages which can be covered to help prevent excess heat loss during this phase.

Collars being used weigh 170g, they will only be applied up to 10 badgers where they are found to equate to not more than 2% of the animal's body weight. Badgers chosen for collaring will be those that meet the required weight and neck: head circumference measurements for a suitable fit as mentioned. Collars are designed to drop off naturally in 6-8 months.

If faecal samples are required, then preference will be given to using faecal samples available from the cage rather than using swabs or enemas.

Badgers will be released from the location of their capture (i.e. within their normal social group environment) with cages being placed in shaded areas as much as possible.

Specific training in the techniques to be used will be undertaken by all personal licence holders and standard operating procedures will be in place. Veterinary surgeons will be available and have oversight of all procedures during trapping weeks so animal suffering and welfare assessment will be covered by Animals (Scientific Procedures) Act 1986 (ASPA) and Royal College of Veterinary Surgeons (RCVS) guidelines.