Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted January – June 2020 that require a retrospective assessment

Contents

1. ADME Studies for Agrochemicals and Veterinary Medicines

Project duration

5 years 0 months

Project purpose

- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Data, Veterinary, Food, Safety, Agrochemical

Animal types	Life stages
Beagles	adult
Rats	adult
Sheep	adult
Chickens	adult
Goats	adult
Pigs	adult
Rabbits	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Uses cats, dogs or equidae

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of this project is to provide a service to Agrochemical and Veterinary companies by generating ADME (Absorption, Distribution, Metabolism and Excretion) and nature of the residue data on candidate test compounds to support the data package to the relevant UK, US, EU and Japanese regulators.

A retrospective assessment of these aims will be due by 24 October 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The ADME (Absorption, Distribution, Metabolism and Excretion) services offered to the Veterinary Medicines and Agrochemicals industries can be divided into studies conducted in laboratory animals to aid interpretation of data from toxicology studies and studies conducted in food-producing animals to identify the nature of the residue in products (e.g. meat, milk, eggs) consumed by humans. These studies are required by regulatory authorities as part of the approval process for Veterinary Medicines and Agrochemicals. Studies required by regulatory authorities to investigate the ADME Properties of Veterinary Medicines and Agrochemicals in laboratory animals are specified and detailed in regulatory guidelines (Ref 1). These studies are conducted in rats.

Critically these studies demonstrate whether the rodents used in toxicology studies produce the same metabolites as are found in residues in the edible tissues of food-producing animals. This information helps to underpin human safety by supporting the data generated from the toxicology studies.

Information on the ADME properties of a test compound aids the understanding of the mechanism of toxicology. Basic pharmacokinetic parameters determined from these studies will also provide information on the potential for accumulation of the test compound in tissues and/or organs and the potential for induction of biotransformation as a result of exposure to the test substance. These data can be used to assess the adequacy and relevance of the extrapolation of animal toxicity data (particularly chronic toxicity and/or carcinogenicity data) to human risk assessments.

In the case of veterinary medicines for the treatment of companion animals, information is required on the ADME properties of each treatment, including metabolites formed, to safely perform acute and repeat dose toxicity studies in the target species and dictate what dose levels to set. As with all medicinal drugs and chemicals it is a requirement to assess the accidental acute exposure in humans as well as understanding any impacts on the environment if the product were to be disposed of incorrectly. These requirements are specifically required to answer EU Directive 2004/28/EC (Amendment of EC Directive 2001/82/EC) Community code relating to veterinary medicinal products which summarises the requirement for this data.

In vivo dermal penetration studies are used as part of operator exposure assessments. Dermal penetration studies in the rat are required to assess the exposure and safety of agricultural workers who work directly with agricultural products as per OECD 427 (Objective 3).

Studies required by regulatory authorities to identify the nature of the residue resulting from use of Veterinary Medicines and Agrochemicals in food producing animals are also specified in detailed regulatory guidelines (Ref 2).
These studies are required to be conducted in ruminants (usually the goat) and poultry (usually the domestic chicken) for Agrochemicals and relevant target species (e.g. pig, sheep) for Veterinary Medicines.

The diet of food-producing animals may contain pesticide residues. It is therefore important to understand what happens to this material in the animal's body and the composition of the terminal residue in the animal products (meat, milk and eggs) that will be consumed by humans. Complete residue detection methodology and residue quantification data can then be developed. Based on the results of the characterisation and/or identification studies, the chemical definition of the total toxic residue (TTR) can then be proposed. All components of the TTR will normally be included in the tolerance expression for the pesticide and residue analytical methods are developed for all components of the TTR.

The human food safety evaluation of veterinary medicines helps ensure that food derived from treated food-producing animals is safe for human consumption. As part of the data collection process studies are required which assess the quantity and nature of residues in food derived from animals treated with a veterinary drug. These metabolism studies provide data on (1) the depletion of residues of concern from edible tissues of treated animals at varying times after drug administration, (2) the individual components, or residues, that comprise the residue of concern in edible tissues, (3) the residue(s) that can serve as a marker for analytical methods intended for compliance purposes (i.e. monitoring of appropriate drug use) and (4) the identification of a target tissue or tissues, as applicable to national or regional program's.

The programme of work outlined in Protocol 03 is designed to determine whether treatment with the candidate monoclonal antibody is able to reduce the activation of the immune system both in response to an antigen to which the dog was previously exposed to (antigen recall) and to a novel antigen (de novo response). To induce antigen recall, an antigen against which the dog was vaccinated can be used, such as canine distemper virus (CDV) antigen which is present in the DHP vaccination the dogs received at 2-3 months of age. De novo immune response can be effectively induced by injection with keyhole limpet hemocyanin (KLH) protein, which is a very immunogenic but safe protein also used in human clinical trials.

Antigen recall studies usually measure systemic reaction after injection by determining T cell activation and antibody production in blood. In the present work we also aim to investigate the ability of our drug to reduce local inflammation. For this reason, we envisage to perform intradermal injection of the antigens and to collect skin biopsies to evaluate the local immune response.

All animal studies are conducted in a facility which operates in compliance with International Good Laboratory Practice Standards (Ref 3).
The following key references support the need for the work related to this project:
Reference 1: Guidelines describing studies to investigate the ADME properties of test compounds in laboratory animals:

Agrochemicals

The Organisation for Economic Co-operation and Development (OECD) Guideline 417 – Toxicokinetics July 2010

The Organisation for Economic Co-operation and Development (OECD) Guideline 427 – Skin Absorption In-Vivo Method, Adopted 2004

EU - Commission Regulation European Union (EU) No 283/2013 March 2013 Section 5.1

US - Office of Prevention, Pesticides & Toxic Substances (OPPTS) Guideline 870.7485 August 1998

Japan Ministry of Agriculture, Forestry and Fisheries (MAFF) Test Guideline 12-Nousan-8147 23-1 November 2000

Veterinary Medicines

International - International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) GL47 Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Comparative Metabolism Studies in Laboratory Animals (2011)

US – Department of Health and Human Services, Food and Drug Administration, Centre for Veterinary Medicine, General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals: Guidance for Industry (2018)

EU - Regulation (EU) 2019/6 of the European Parliament And Of The Council of 11 December 2018 on veterinary medicinal products

EC Directive 2004/28/EC (Amendment of EC Directive 2001/82/EC) Community code relating to veterinary medicinal products

The rules governing medicinal products in the European Union, Volume 6B Notice to applicant, Veterinary Medicinal Products, Presentation and content of the dossier, 2015. Reference 2: Guidelines describing studies to investigate the nature of the residues in food producing animals:

Agrochemicals

International - Organisation for Economic Co-operation and Development (OECD) 503 Metabolism in Livestock (2007)

US - Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency (OPPTS) Guideline 860.1300 Nature of the Residue – Plants, Livestock (1996)

EU - EC Working Document 7030/VI95 Rev 3 Metabolism and Distribution in Domestic Animals (1997) (detailed guidance on study conduct)

EU - Commission Regulation (EU) No 283/2013 March 2013 Sections 6.2.2 and 6.2.3 (data requirements)

Japan - Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF) 2-4-2 Metabolism in Livestock (2014) (new data requirement)

Veterinary Medicines

International - International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) GL46- Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Metabolism Study to Determine the Quantity and Identify the Nature of Residues (MRK) (2011)

US – Department of Health and Human Services, Food and Drug Administration, Centre for Veterinary Medicine, General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals: Guidance for Industry (2018)

EU - Regulation (EU) 2019/6 of the European Parliament And Of The Council of 11 December 2018 on veterinary medicinal products

Reference 3. Good Laboratory Practice Regulations:

The United Kingdom (UK) Good Laboratory Practice Regulations 1999 (Statutory Instrument No. 3106) and subsequent amendment.

The Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (Paris 1998)

European Commission (EC) Commission Directive 2004/10/EC (February 2004)

What outputs do you think you will see at the end of this project?

Outputs arising from this Project include:

- Confirmation or refutation that laboratory animals used in toxicology studies produce the same metabolites as are found in residues in the edible tissues of food-producing animals
- ADME data following the oral, intravenous and/or dermal dose routes, which will assist in the interpretation of toxicology studies Rate and extent of oral absorption of test compounds
- Determination of PK parameters (C_{max}, T_{max}, AUC, Bioavailability (F)) of test compounds
- Assessment of potential for bioaccumulation of test compounds
- Plasma half-life of test compounds
- Distribution of a test compound in major organs and tissues
- Distribution of a test compound in blood cells
- Determination of Metabolic pathways of test compounds
- Identification of Metabolites of test compounds
- Determination of route and time course of excretion of test compounds and their metabolites
- Investigation of whether and to what extent enterohepatic circulation of test compounds takes place
- Assessment of rate of dermal absorption of test compounds
- Outputs from food-producing animal studies:
- Estimation of total residues of test compounds in the edible livestock commodities, as well as excreta of test compounds

- Identity of the major components of the terminal residue in the edible tissues of test compounds, thus indicating the components to be analysed in residue quantification studies
- Elucidation of metabolic pathways of test compounds
- Provision of evidence for whether or not a residue should be classified as fat soluble
- Demonstration of the depletion of the marker residue upon cessation of drug treatment to the regulatory safe level
- Generation of data suitable for elaboration of appropriate withdrawal periods/withholding times to address consumer safety concerns
- Ultimately these outputs will be used to assess the safety of veterinary products and agrochemicals resulting in only safe products reaching the market.

Who or what will benefit from these outputs, and how?

The principal beneficiaries of the studies conducted under this Project are:

The Veterinary Medicine and Agrochemicals industries as the studies conducted form part of the dossier submitted for regulatory approval of their products.

The general public who benefit from food that has been produced using new safer agrochemicals and veterinary medicines which have a lower impact on the environment.

The farming industry who will benefit from safer, more effective veterinary medicines and crop protection chemicals resulting in healthier animals and higher crop yields.

The general public who benefit from an improved safety assessment process due to product registration/re-registration to ensure that only products which meet modern safety and regulatory standards enter/remain on the market and are assessed using modern experimental/analytical methodologies.

Animals which will benefit from improved health and welfare as a result of more effective veterinary medicines.

How will you look to maximise the outputs of this work?

Subject to approval from Study Sponsors we will endeavour to publish as much information as possible from studies conducted under this Project License.

Species and numbers of animals expected to be used

- Pigs: 20
- Goats: 20
- Sheep: 20
- Domestic fowl: No answer provided
- Rats: 1000
- Rabbits: 50
- Beagles: 80

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using these species of animals as the data they produce is a regulatory requirement for the submission of agrochemicals and veterinary medicines.
Only adult animals will be used.

Typically, what will be done to an animal used in your project?

Typically the following will be done to animals under this license:

- Animals will be singly housed in a metabolic cage for up to 1 day prior to dosing
- Administration of a test substance by the intravenous, oral, subcutaneous, intramuscular or topical routes.
- Following dosing animals will be singly housed in a metabolic cage for the collection of urine and faeces for up to 15 days in the case of pigs, sheep, goats, rabbits and chickens but generally 8 days. Rats maybe singly housed for up to 8 days for the collection of urine, faeces and expired air.

Less typically:

- Rats may have food withdrawn for up to 24 hours prior to dosing but normally no longer than 14 hours.
- Administration of a test substance by the intravenous infusion, intramuscular or intradermal routes.
- The collection of blood samples by direct puncture of a jugular vein after dose administration for dogs, pigs, sheep and goats. Chickens will have blood samples collected by direct puncture of a wing vein and limited to 2 blood samples only. Rats and lagomorphs will have blood samples collected from an indwelling catheter over the first 8 hours following dosing. Samples collected beyond 8 hours post dose will be collected from all species by direct venepuncture. Direct venepuncture is limited to 10 occasions only over any 24 hour period (apart from the chicken).
- Following intra dermal dosing only under Protocol 3, up to 7 small skin biopsies (3 to 5mm) will be taken from the side of each dog whilst anaesthetised, under supervision by the NVS on the pilot study. On the main study up to 4 biopsies maybe collected from the anaesthetised dog under supervision by the NVS.

What are the expected impacts and/or adverse effects for the animals during your project?

We do not expect to see any adverse effects in the studies we perform through investigation of the compound prior to a study being initiated. This involves the understanding of the compounds safety in previous studies so carefully selected dose levels are administered minimising the chances of any adverse effects being seen. Following completion of each project animals will be humanely killed.

The withdrawal of blood samples from superficial vessels, by direct venepuncture, may cause a small period of discomfort as the needle is inserted and blood sample collected. Pressure is applied to the site following collection via cotton wool.

When orally dosing, animals will be restrained and the drug administered directly into the stomach via a syringe and gavage tube. This may cause a period of discomfort whilst the procedure is being performed.

Intravenous doses are administered directly into a cephalic vein generally over a 1 minute period whilst being continually held by a technician.

Intravenous infusion administration, in dogs only, will be given by a calibrated infusion pump into a cephalic vein via an indwelling catheter for a maximum period of 30 minutes within the NC3R recommended guidelines on dose volumes. All dogs will be held by a license holder during the infusion.

When a study requires the individual collection of urine and faeces animals will be singly housed in a metabolic cage to enable the separate collection of these samples. During this period the animals may become quieter and less active compared to normal due to the change in type of housing and no direct contact with other animals. To mitigate this the animals will be housed adjacent to each other so they have visual contact at all times. The time period animals are allowed to be singly housed in metabolic cages is strictly controlled. Where possible we use environmental enrichment such as playing background music and hanging toys.

Under Protocol 03:

The test substances are novel fully canine monoclonal antibodies. Canine monoclonal antibodies are currently being used in dogs (such as Cytopoint and Librela) and have demonstrated a very safe profile. As such we do not anticipate significant side effects from our fully canine monoclonal antibodies, or any immune reaction against them. As no canine antibodies exist at present that target the same antigen in dogs it is hard to predict the target mediated adverse effects. A human monoclonal antibody for the target of the same antigen in humans has been developed and showed a very safe profile in both phase I and II clinical trials, suggesting that the test substances should be safe and well tolerated. If in the very unlikely case of anaphylactic reaction an injection of epinephrine may be administered

Procedural impacts:

Intramuscular, subcutaneous, or intradermal injection of CDV containing vaccine (i.e. DHP vaccine) is not anticipated to produce any adverse effect as it is routinely used to immunise dogs.

Intramuscular, subcutaneous, or intradermal injection of KLH protein is not anticipated to produce any adverse effect as it has been safely used in human clinical trials and there are previous reports of its use in dogs.

All punch biopsies will be performed under the supervision of our NVS and the dogs anaesthetised prior to the procedure being performed. Punch biopsies will cause mild pain to the animal and can be the cause for infection. To minimise this risk and reduce animal suffering, we will take precautions as listed below. Before this procedure is conducted the skin around the area of interest will be shaved, cleaned and a general anaesthetic applied to minimise any pain caused to the animal. Following the procedure an antiseptic cream may be applied to the biopsy site which may be repeated if necessary. Pain management may also include administering oral painkillers (such as non steroid antiinflammatory drugs), if required, and prescribed in consultation with the NVS and NACWO. Cortisone

and other drugs that affect the immune response should be avoided as they will interfere with the experimental results. The biopsy area maybe covered following collections to minimise damage caused by scratching etc. The wound will be visually inspected at least twice daily to assess signs of redness or swelling. Antibiotics, both topical and oral may be administered if deemed necessary by the NVS or NACWO. No animal will have more than 7 biopsies collected.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Approximately 50% of rats will experience a moderate severity limit and 50% a mild severity limit.

All pigs, sheep, goats, rabbits and chickens will experience a moderate severity limit.

Approximately 50% of dogs will receive a moderate severity and 50% a mild severity.

What will happen to animals at the end of this project?

- Killed
- Kept alive

A retrospective assessment of these predicted harms will be due by 24 October 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Various international regulatory guidelines specify the generation of safety data in two species, one of which must be a rodent. The use of rodents in safety evaluation has been generally accepted by scientific and regulatory bodies, because of the ease of breeding and availability, straightforward husbandry, growth rate, ease of handling under experimental conditions and broad physiological similarities to man. Extensive background data is available in any particular procedure to enable meaningful statistical evaluation to be performed.

In support of the regulatory guidelines, ADME studies in animals:

- confirm whether toxicology studies produce the same metabolites as those found in edible tissues of food producing livestock provide PK statistics (e.g. C_{max} and T_{max}) to

assist in the interpretation of toxicology studies estimate the total residues in the edible livestock and excreta identify the major components of the terminal residue in the edible tissues, indicating the components to be analysed in residue quantification studies
- demonstrate the depletion of the marker residue upon cessation of drug treatment to regulatory safe level

Which non-animal alternatives did you consider for use in this project?

Various *in vitro* and *in silico* evaluations are being used to provide predictive information and where such information is available this leads to the replacement of animals. For example, in some situations, the use of ethically procured human skin can provide information directly applicable to humans without recourse to initial evaluation in animals.

Why were they not suitable?

Despite the advance in non-animal methods it is still a requirement to use animals where there are no viable alternatives in order to produce information on the safety of Agrochemicals and Veterinary Medicines for Regulatory authorities.

A retrospective assessment of replacement will be due by 24 October 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

As a Contract Research Organisation the number of animals used will primarily be based on the number of studies performed for clients. The number of animals used can then be estimated according to the regulatory guidelines the studies are being run to meet.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have a vast amount of knowledge with Principal scientists, Team leaders and Study directors who have extensive experience of designing studies of this type, with the aim of keeping the number of animals used to a minimum. All of these people within the organisation have statistical expertise as this is one of their primary functions when discussing and interpreting data.

We always use the minimum number of animals required on a study whilst still providing robust scientific data which will be accepted by international guidelines for the safety of agrochemicals and veterinary products in man.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Prior to any study initiating we actively source relevant information regarding the test item which will have an impact on the study design and number of animals used. If group sizes of >3 are requested, our AWERB (Animal Welfare and Ethical Review Body) will rigorously check the study design to ensure the use of larger groups sizes is justified and if this cannot be, a group size of n=3 will be used.

Where possible in rodent studies, and as long as the burden is not too great for the animal, each animal will have multiple outputs to reduce the overall number of animals required on a study. For example where animals are used for the collection of excreta, following the last collection the animals would be used for blood collection under general anesthesia which would not increase the overall burden on the animal but reduce the number of animals required for terminal blood collection. Apart from the blood collection under general anesthesia no additional burden would be placed upon the animals.
When the animals are procured generally a certain number of additional animals (~5%) are procured for each study in case of any miss-dosing, such as part of an intravenous dose going subcutaneous or part of an oral dose refluxing into the mouth. Prior to an ADME (Absorption, Distribution, Metabolism and Excretion) experiment starting various in-vitro and in-vivo studies could have been performed which would highlight any need for increased group sizes to prove significance in the data provided and ensure the study objectives are met.

Where group sizes requested are more than generally used, we will consult with a statistician in order to ensure that the study is conducted with the minimum number of animals whilst still producing scientifically significant data.

The group sizes above are also dictated by the individual compound characteristics such as solubility.

For example a solution dose will yield much tighter results compared to a suspension dose given orally.

For the smallest possible group size great care is taken to ensure all samples are collected exactly on time and to a high standard. The exact time of sampling is calculated to ensure all samples are collected on the minute to help ensure the data produced is as significant as possible reducing the variability between animals.

Examples of strategies reducing the total number of animals used:

The continuing drive for repeated serial blood sampling in rats, as opposed to a composite design, reducing the total number of rats used in ADME studies.

Obtaining multiple samples from a single animal, e.g. in ADME studies using the 7 day balance excretion animals (collection of urine and faeces) for the 7 day QWBA (Quantitative Whole Body Auto Radiography) phase - reducing the number of animals used on the QWBA phase.

A retrospective assessment of reduction will be due by 24 October 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The choice of species and models used in this Project are effectively directed by Regulatory International Guidance's and/or relate to prior safety models and ensure that the species used are the most refined for the intended purpose.

The international guidance for safety assessment in agrochemicals mandates two mammalian species including at least one non-rodent species. In practice this means rat for the rodent species. The use of pig, sheep goat or rabbits is to help determine human exposure via the food chain. In the case of veterinary medicines the rat will be used as the rodent species and the non rodent species will generally be the target species receiving the end medicine which will either be the pig, sheep, rabbit or chicken.

- withholding of food for a single period in rats only, generally not more than 12 hours, but not exceeding 14 hours

- We withhold food for the minimum period only, which normally involves a PIL holder coming back to in the evening to withdraw food as this gives rodents part of their dark cycle to feed, which causes the least distress to the animals.

- withdrawal of small blood samples of blood from a superficial blood vessel in rodents and large animals and/or via an indwelling cannula (rats and rabbits only)

If multiple samples are being collected in the rat or rabbit an indwelling cannula will be inserted into a lateral tail vein or marginal ear vein in the rabbit to ensure the least number of vene punctures are performed. The withdrawal of blood samples from a superficial vessel is a quick and easy method to provide blood samples and we have extensive experience of performing this procedure to a very high standard. The minimum amount of blood required will be collected at all times to the recommended volumes. The indwelling cannula used in the rat and rabbit will be primed with heparinised saline following each blood sample collection to maintain patency and will not be left in place overnight.

- Confinement in a metabolism cage (large animals up to 15 days only) or metabowl (rodents up to 8 days)

There is currently no other option that causes less harm and uses less number of animals to collect excreta from animals at defined time periods. Rats and large animals will be singly housed but visual access to other animals will be insured by housing the metabowls/metabolism cages side by side in the same room. If any clinical signs are observed in any animals whilst in the metabowls/metabolism cages the NACWO/NVS will be consulted and if required the animals killed. Rats in metabowls will be checked at least twice daily when expired air is collected as the possibility of asphyxiation is increased due to the pumps relying on an electrical source. All pumps drawing air through the metabowls have a backup UPS battery supply, which lasts for at least three hours, which is adequate time for a PIL holder to be called out to remove the air traps from the metabowls.

Administration of substances by one of the following routes:

Intravenous injection, Infusion via cannula, intramuscular, Intraperitoneal injection, cutaneous, subcutaneous injection
Irritation caused by injection will be avoided as much as possible by injecting small volumes and administering one dose only via all routes. If clinical signs are observed, and deemed necessary, animals will undergo an evening health check.

Oral gavage

Damage to the mouth or osophagous be minimised by the use of flexible polypropylene gavage catheters and continuous monitoring of the animal. If clinical signs are observed, and deemed necessary, animals will undergo an evening health check

Topical application

After topical application, particular attention will be paid to the possibility of inflammation of the dose site. The smallest area of skin will be used for administration whilst adhering to the regulatory guidelines on the defined area to be dosed.

Killing via a Schedule One method

This is terminal procedure carried out by highly trained licencee's and as such will cause no distress apart from the anesthetising procedure in the rat or intravenous administration of substances causing death in large animals, domestic fowl and rabbits.

Killing via non Schedule One method

This is a terminal procedure specifically used only on rats. It involves the sacrifice of an animal via rising concentration of CO_2 then once confirmed dead, immersion in a liquid mixture of cardice and hexane (ca.-120 degrees C).

Why can’t you use animals that are less sentient?

We strive to use the least sentient animal species where possible whilst still meeting the objectives of the study and adhering to the International Regulatory guidelines the study is being run to meet. Ultimately the species used is governed by the requirements of regulatory authorities that the client is conducting the work to meet.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

A system of continuous refinement of procedures operates such that the least severe option is maintained, as illustrated in the following examples:

Using large fun tunnels, chewing blocks and fruity chews for rabbits

The use of aspen balls for rats

The continual use of a simple blood vessel cannulation procedure in rats and rabbits to minimise serial venepuncture.

The use of micro sampling in rats reducing the volume of blood required when multiple sampling.

Ensuring the minimum quantity of blood is collected to achieve scientific objectives

There are no severe protocols in this programme of work with no surgical procedures being performed under this license.

If any clinical signs are observed which are manageable and do not exceed the project license evening checks will be performed to ensure the animal does not deteriorate and if it does, the appropriate intervention can be made.

We always strive to adhere to the highest possible welfare standards for the animals, for example the use of high top caging for the rats to ensure they can fully rear up.

We are a GLP accredited facility and as such conforms to the highest possible international standards with our internal Quality Assurance department conducting routine (~3 months) inspections of nonprocedural work such as weighing of the animals and monitoring their cage cleaning.

We will also request prior compound data from the client that could aid dose levels and species selection. Prior *in vivo* and *in vitro* data will be used to select the most appropriate species to meet the scientific objective. The overriding decision will be to use the lowest order species first such as the rat prior to using any higher order species such as the pig, sheep or goat. If limited rodent data is available we will ensure that the dose levels selected minimise potential clinical signs which may be seen in the sheep, rabbit, chicken, goat or pig.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Guidelines describing studies to investigate the ADME properties of test compounds in laboratory animals:

Agrochemicals
International - Organisation for Economic Co-operation and Development (OECD) Guideline 417 - Toxicokinetics July 2010
International - Organisation for Economic Co-operation and Development (OECD) Guideline 427 - Skin absorption, In-Vivo method. Adopted April 2004
EU - Commission Regulation (EU) No 283/2013 March 2013 Section 5.1
US – Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency (OPPTS) Guideline 870.7485 August 1998

Japan – Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF) Test Guideline 2-4-2 Metabolism in Livestock (2014) (new data requirement)
Veterinary medicines International - International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) GL46- Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Metabolism Study to Determine the Quantity and Identify the Nature of Residues (MRK) (2011)
US – Department of Health and Human Services, Food and Drug Administration, Centre for Veterinary Medicine, General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals: Guidance for Industry (2018)
EU - Regulation (EU) 2019/6 of the European Parliament And Of The Council of 11 December 2018 on veterinary medicinal products
Guidelines describing studies to investigate the nature of the residues in food producing animals:
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International - Organisation for Economic Co-operation and Development (OECD) Guideline 503 - Metabolism in Livestock (2007)
US – Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency (OPPTS) Guideline 860.1300 Nature of the Residue - Plants, Livestock (1996)
EU - EC Working document 7030/V195 Rev 3 Metabolism and Distribution in Domestic Animals (1997) (detailed guidance on study conduct)
EU - Commission Regulation (EU) No 283/2013 March 2013 Sections 6.2.2 and 6.2.3 (data requirements)
Japan – Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF) Test Guideline 2-4-2 Metabolism in Livestock (2014) (new data requirement)
Veterinary Medicines
International - International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) GL46- Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Metabolism Study to Determine the Quantity and Identify the Nature of Residues (MRK) (2011)
US – Department of Health and Human Services, Food and Drug Administration, Centre for Veterinary Medicine, General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals: Guidance for Industry (2018)
EU - Regulation (EU) 2019/6 of the European Parliament And Of The Council of 11 December 2018 on veterinary medicinal products
Good Laboratory Practice Regulations:
The United Kingdom (UK) Good Laboratory Practice Regulations 1999 (Statutory Instrument No. 3106) and subsequent amendment.
The Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (Paris 1998)
European Commission (EC) Commission Directive 2004/10/EC (February 2004) All studies will be performed to the standards of GLP.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

All PIL holders and myself as Project license holder go on relevant training/refresher courses annually and keep up to date with reduction and refinements by attending NC3R/RSPCA workshops or equivalent as part of our continual development programme. This information is then shared among the animal care staff.

A retrospective assessment of refinement will be due by 24 October 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

2. Antigen targeting and delivery

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The overall goal of this research is to define how different configurations of antibody-antigen complexes, or Fc-antigen fusions, can lead to antigen presentation and T cell responses in cancer and autoimmunity.

A retrospective assessment of these aims will be due by 25 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cancer is the cause of 25% of all deaths in the UK, with a prediction that by 2020, almost 50% of people will be affected by this disease. Despite the development of better diagnostic and screening methods, the incidence of cancer continues to increase and many cancers are diagnosed late. Although surgery followed by chemotherapy is frequently used to treat disease at diagnosis, this is often not curative due to incomplete removal of tumour cells that lead to spread i.e. metastatic disease. Similarly, autoimmune diseases affect about 6% of people in the UK and can have devastating consequences, in addition to causing death in a significant proportion of patients. Current treatments for autoimmunity frequently have undesirable side effects and, for some types of disease, have limited efficacy. Consequently, a detailed mechanistic understanding of the factors that result in anti-cancer immune responses or the silencing of damaging, self-reactive responses in autoimmunity is highly desirable. Our proposed research programme seeks to address this need.

What outputs do you think you will see at the end of this project?

The proposed programme of research comprises both basic mechanistic analyses and translational studies. It is expected to lead to new insight into how cells called macrophages can affect the behaviour of an important immune cell subset called T cells. The macrophages are able to modify proteins called antigens into a form that is recognized by T cells, using a process called antigen presentation. A substantial part of our programme will involve the use of different configurations of antigens, including antigens bound to proteins call antibodies, that are expected to show distinct behaviour in macrophages. This distinct behaviour is in turn expected to lead to varying responses by T cells.

Although T cells can contribute to anti-cancer effects, they can also attack a person's own components and lead to autoimmune diseases such as multiple sclerosis and arthritis. The predicted outcome of our five year study is that we will generate mechanistic information concerning how T cells can be activated to kill tumour cells (immune activation), or silenced to reduce their detrimental effects on self- components (tolerance).

Although we expect that our studies could ultimately lead to translation of possible therapeutics to the clinic, this is predicted to fall outside the five year time frame of the study. In this context, we have extensive experience in outlicencing of technologies that we have developed to biopharma that have to date resulted in late phase II/phase III trials using antibody engineering technologies developed in our laboratory.

Data from the planned studies is expected to be of interest to other researchers, clinicians and biopharma. The outcomes of our studies will be disseminated through publications and presentations at conferences. We will also participate in public outreach activities to inform the general public about our research and its relevance to human disease.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Our findings during the five year research programme are expected to result in new insight concerning important immunological processes that relate to how antigens can be configured to result in T cell activation or silencing. As such, the research outcomes during this time period are expected to be of broad general interest to researchers (in both academia and biopharma) and clinicians. In the longer term we plan to be able to translate our findings to the development of antigen-based therapeutics that can be used to treat

people who suffer from autoimmune disease or cancer. This translation is expected to fall beyond the five year time frame of the research programme.

How will you maximise the outputs of your work?

Outputs will be maximized by careful and rigorous experimental design/analysis, combined with efficient running of the laboratory.

Species and numbers of animals expected to be used

- Mice: 4976

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

The typical experience of a mouse is as follows:

For pharmacokinetic experiments in normal mice (mild severity, 100% mice): mice will be injected with radiolabelled protein and the levels of radiolabel in blood (16 total samples and a maximum of 3 samples being taken per day) and whole body (18 total measurements and a maximum of 3 measurements being taken each day) determined for up to 14 days.

For pharmacokinetic experiments in tumour-bearing mice (mild severity, 95% mice; moderate severity, 5% mice): mice will be injected subcutaneously with tumour cells, and tumour size monitored using calipers. When the tumours reach a moderate size, mice will be injected with radiolabelled protein and treated as above. These experiments will last for a maximum of 30 days (including tumour growth phase).

For immunological assays in tumour-bearing mice (mild mild severity, 95% mice; moderate severity, 5% mice): mice will be injected with tumour cells, and tumour size monitored using calipers. When the tumours reach a moderate size, mice will be injected with engineered proteins of different configurations. 1-10 days later, organs and tissues will be isolated from the mice. These experiments will last for up to 30 days.

For immunological assays in mice with MOG-specific immune responses (mild severity, 97-98% mice; moderate severity 1-2%; severe severity, 1% mice): mice will be immunised with peptide (passive immunisation model) or protein antigen (active immunisation model). Peptide-immunised mice will be injected with MOG-specific antibodies on day 10-15 following immunisation. 0-1 day following MOG- specific antibody delivery, or 9-15 days post-immunisation with MOG protein, mice will be injected with engineered proteins of different configurations. 1-10 days later, organs and tissues will be isolated from the mice. These experiments will last for up to 26 days.

For immunological assays in mice with autoimmune disease (EAE) (moderate severity, 35% mice; severe severity, 65% mice): mice will be immunised with peptide or protein

antigen, followed by injection of a protein called pertussis toxin on the same day (day 0) and two days later. The disease score of the mice will be assessed daily, and mice will be monitored three times per day when any mice reach a mild-moderate disease score. Peptide-immunised mice will be injected with antibodies when they reach a mild-moderate disease score of 1-2 (day 9-15). 0-1 day following this, or when protein- immunised mice reach a mild-moderate disease score (day 9-15), mice will be injected with engineered proteins of different configurations. 1-10 days later, organs and tissues will be isolated from the mice. These experiments will last for up to 26 days.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

In a subset of our proposed experiments, mice will be injected with tumour cells under the skin. These experiments will last for up to a maximum of 30 days, with the tumours growing from being undetectable to measurable during this time. Mice will be carefully monitored and, typically, the tumours will not grow to a size where they interfere with the normal behaviour of the mice such as their ability to move, eat or drink. We therefore do not expect to observe body weight loss. In the unlikely event that we observe effects of the tumour on the behaviour of the mice, or if the tumours ulcerate, the mice will be humanely killed. In addition, for studies investigating the behaviour of the antibody/antigen/Fc-antigen fusions of different configurations in tumour-bearing mice, the short-term nature of the experiments and regulated procedures that will be used are expected to primarily result in effects of mild severity, with a small percentage (around 5%) of mice showing moderate effects. Typically, therapeutic antibodies and antibody-based proteins are well tolerated and will be used at doses that are expected to be well below the maximum tolerated dose, which is the highest dose that can be delivered without observing significant, undesirable side effects.

For anaesthesia, we will follow current, best practice methods and do not expect the mice to suffer adverse events. For non-invasive methods such as delivery of antibodies/antibody-based agents by intravenous injection, mice will not be anaesthetised since these procedures result in only transient pain and/or distress.

For harvesting of blood samples from mice, we will use volumes that are substantially lower than those likely to cause adverse effects such as anaemia. Mice will be bled using best practice methods by trained personnel. Based on many years of using similar protocols, we do not expect the effects to be greater than the mild level. Wherever possible, we will use whole body counting of mice, as an alternative to bleeding, since this procedure involves placing the mouse in a cylindrical body counter for around one minute, whilst still allowing some movement of the mouse. Whole body counting involves placing the mice in a cylindrical container (similar to an MRI machine) for several minutes and results in minimal disturbance to the welfare of the mouse.

For experiments involving the immunisation of mice with MOG peptide or protein in adjuvant, following by transfer of MOG-specific antibodies for peptide-immunised mice, we expect to observe skin lesions at the injection sites for a low percentage of mice (2-3%) that resolve within 1-2 days. In about 1% mice, these skin lesions persist and these mice will be humanely killed.

For experiments in which the autoimmune disease called experimental autoimmune encephalomyelitis, that involves paralysis similar to that seen in multiple sclerosis patients, is induced in mice, about 35% of total mice used in two different models are expected to

get low grade disease i.e. limp tail, partial hind limb weakness. These effects will be classified as of moderate severity. In about 65% of mice, we expect to see higher disease activity that includes severe or complete hind limb paralysis, and in the majority of mice, this will not last for more than 1-4 days. In a total of about 20% mice used in both disease models, we may see moderate disease that lasts for longer or is more severe and these mice will be humanely killed.

All mice will be humanely killed when the experimental goal or, if sooner, humane endpoint has been reached.

Expected severity categories and the proportion of animals in each category, per species. What are the expected severities and the proportion of animals in each category (per species)?

The expected severities for each type of experiment and mouse numbers over the five year study period are:

Pharmacokinetic studies in normal mice: mild severity (100% mice).

Pharmacokinetic studies in tumour-bearing mice: mild severity (95% mice); moderate severity (5% mice).

Immunological studies in tumour-bearing mice: mild severity (95% mice); moderate severity (5% mice).

Immunological studies in mice that have been immunised with autoantigen (MOG): mild severity (97- 98% mice); moderate severity (1-2% mice); severe severity (1% mice).

Immunological studies in mice with autoimmune disease (EAE): moderate severity (35% mice); severe severity (65% mice).

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 25 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our goal is to replace animals with in vitro methods wherever possible. We will therefore carry out extensive analyses of the engineered antigen delivery vehicles using in vitro assays to identify promising candidates for use in mice. Unfortunately, the complexity of

the persistence of an antigen delivery vehicle in the blood circulation cannot be modelled with in vitro systems. In addition, investigation of the immune responses that result from the delivery of antigen delivery vehicles in mice with tumours or autoimmune disease cannot be modelled by in vitro systems.

What was your strategy for searching for non-animal alternatives?

We have considered using in vitro methods (e.g. cell culture, microscopy, binding analyses) and will used these whenever possible to identify lead candidates for in vivo testing. However, whole body pharmacokinetic behaviour and immune responses cannot be replaced with in vitro systems due to the complexity of the body.

Why were they not suitable?

Cell culture-based methods cannot be relied upon to predict immune responses and pharmacokinetic behaviour in vivo. For example, the distribution of a protein to a tumour cannot currently be modelled by in vitro cell culture models. In addition, diseases such as cancer and autoimmunity, that involve multiple cell types and complex environments, cannot be modelled by cell culture models.

A retrospective assessment of replacement will be due by 25 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

A major contribution to our experimental design will be consideration as to how the animal numbers can be reduced. We will use inbred strains of mice (i.e. with the same genetic makeup) for all of our experiments to reduce the variability that would typically be expected with outbred strains (that are not genetically the same). This results in a need for lower mouse numbers. Our aim is to use the minimum number of mice that we can to obtain statistically robust results that are reproducible across experiments. We will use both our prior experience in carrying out the proposed experiments, combined with power analyses, to determine the numbers of mice that we need for each experiment to draw reliable conclusions. In addition, we will carry out smaller, pilot experiments with low numbers of mice, to define tumour growth rates etc. prior to expansion to larger experiments if we have not prior experience with the tumour model. Nevertheless, the use of tumour cell lines that are well validated in our studies or those of others will form the basis of many of our analyses, and this is expected to contribute to a need for lower numbers of mice. The immunisation and autoimmune disease models that we plan to use are well characterised and will not need pilot experiments of this type.

Where possible, we will use data from experiments that are associated with lower severity of adverse effects to reduce the numbers of mice that we use in higher severity procedures. An example of this is our plan to carry out initial experiments with engineered proteins in mice that have been immunised to induce an autoreactive immune response prior to experiments with a subset of lower numbers of these engineered proteins in mice that are expected to show adverse effects of higher severity due to the induction of autoimmune disease. The choice of this subset of proteins will be informed by the lower severity experiments in immunised mice.

Technologies such as whole body counting for pharmacokinetic analyses also result in lower numbers of mice since they allow longitudinal follow-up of the same mice over time. These alternatives avoid the need for increases in mouse numbers due to limitations of the number of blood samples that can be taken from the same mouse. However, for determination of blood levels of a potential antigen delivery vehicle, we need to collect multiple blood samples. Our experience in carrying out pharmacokinetic experiments is that longitudinal sampling of the same individual mice leads to more reliable results than cycling blood collections between different groups of mice. This results in a need to use substantially lower numbers of mice.

We will also make every effort to decrease experimental bias and minimise experimental variability. An example of this will be if the humane end points are based on an assessment of the condition of the animal, such as during EAE experiments, an experienced and blinded animal technician will be asked for their assessment. Objective measurements such as tumour measurement, whole body counts etc. generally do not need blinded observers. Allocation of mice to experimental groups is carried out by a technician in the breeding colony prior to transfer to the experimental housing and is therefore not likely to be biased. Related to this, we use age and sex-matched mice that have been bred in the same housing for experiments to minimise variability.

Statistical considerations

Based on earlier experiments that we have carried out, we have used power analyses to determine optimal sample size for the pharmacokinetic analyses (using the PS: Power and Sample Size Calculation programme: www.biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize):

Pharmacokinetic analyses (Protocol 1)

Using data from earlier experiments, we expect that the smallest difference in means between test and control groups that we will need to be able to detect is 20 (hours) with a common standard deviation of 15. Based on a two sided t-test using a power analysis with a level of significance of 5% with 80% power, the number of mice per group is 7. We typically carry out repeat experiments to ensure reproducible results, and based on around 25 years of experience with these assays, will use 5 mice per group.

The total number of mice that we estimate we will need for these experiments is predicted to be:

Per year, we expect to generate 8 antibody/antigen/Fc-antigen fusions for testing in mice (normal and tumour-bearing). We will typically test 2 proteins per experiment with one control antibody (i.e. 5 mice per group, resulting in 15 mice per experiment). Hence total number of mice used per year is estimated to be:

15 mice/experiment x 4 experiments x 2 repeats x 2 mouse types (with or without tumours) = 240.

Hence, over 5 years the total number of mice is expected to be 1200.

Immunological, microscopy and immunohistochemical analyses (Protocols 2, 3 and 4)

Based on our extensive experience assessing serum antibody levels and T cell responses in mice, we will use 3 mice/treatment group for these experiments to determine antibody responses combined analyses of immune cells in tumours (Protocol 2) or the central nervous system (Protocol 3) following the use of the engineered antibody/antigen/Fc-antigen fusions. T cells and antigen presenting cells will also be isolated from lymphoid organs such as spleen and lymph nodes (Protocols 2, 3 and 4).

Similarly, 3 mice/group will be used for tumour or CNS isolation from tumour-bearing mice or mice with EAE, respectively, for use in immunopeptidome, microscopy or immunohistochemical analyses. A subset of experiments will also be carried out involving the use of both antigen delivery vehicles and immunotherapeutic antibodies. We usually carry out repeat experiments to ensure reproducible results. For each of protocols 2 and 3, the numbers of mice needed have been estimated as follows:

Protocol 2: Assessing immune responses in tumour-bearing mice

We plan to test 8 different antibody/antigen/Fc-antigen fusions per year in mice with tumours, and will use 2 delivery vehicles and 2 controls per experiment with 3 mice per group. Also, two different tumour models expressing different antigens will be used. We will start each experiment with 15 mice, since we need to select 12 from these with matched tumour sizes. Hence total number of mice used per year is estimated to be:

15 mice x 4 experiments per year x 2 (immunological responses or microscopy/immunohistochemistry and immunopeptidome analyses) x 2 tumour models x 2 repeats = 480 mice.

Hence, over 5 years the total number of mice is expected to be 2400.

Protocol 3: Assessing immune responses in mice with EAE

We plan to test 4 different configurations of antibody/antigen/Fc-antigen fusions per year in mice with EAE, and will use 2 delivery vehicles and 2 controls per experiment with 3 mice per group. We will start each experiment with 20 mice, since we need to select 12 from these with matched EAE scores and several mice in control groups (with higher disease activity) may need to be euthanised during the course of the experiment. Hence total number of mice used per year is estimated to be:

mice/experiment x 2 experiments per year x 2 (immunological responses or microscopy/immunohistochemistry and immunopeptidome analyses) x 2 repeats = 160 mice.

Hence, over 5 years the total number of mice is expected to be 800.

Protocol 4: Assessing immune responses in autoantigen-immunised mice

We plan to test 8 different configurations of antibody/antigen/Fc-antigen fusions per year over a 4 year period in immunised mice, and will use 2 delivery vehicles, an average of 5

controls per experiment and two time points per experiment with 3 mice per group. Hence total number of mice used per year is estimated to be:
mice/experiment x 4 experiments per year x 2 time points x 2 repeats = 336 mice.
Hence, over 4 years the total number of mice is expected to be 1,008.
The projected use of mice in this application is based on a combination of our past experience in using mice to assess the in vivo behavior of antibodies and antibody-based therapeutics, combined with power analyses such as those given as examples above. The numbers are also based on expected numbers of laboratory personnel and potential antibody/antigen/Fc-antigen fusions that we plan to generate.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

We have used power analyses and our prior experience to use the minimum number of mice that we expect to be necessary to give us reliable data (see section above). If necessary, we will seek advice of statisticians or use online resources (e.g. https://www.nc3rs.org.uk) to determine the numbers of mice to use in each experiment to obtain useful and statistically robust data.

What other measures apart from good experimental design will you use to minimise numbers?

When indicated, we will carry out pilot studies using smaller mouse group sizes etc. prior to performing larger experiments. Advice from statisticians will be sought if necessary to optimise experimental design. All work will be carried out in accordance with the ARRIVE guidelines.

A retrospective assessment of reduction will be due by 25 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.
Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

The overall goal of this project is to investigate the effects of using different engineered proteins (called antibodies, antigens or Fc fusions) on immunological responses involving cells called T cells. Mice are the least sentient of the species that are appropriate for this research and have been widely shown to be suitable preclinical models for immunological studies. The mouse models of cancer and autoimmunity that we plan to use have been used extensively and are well validated as appropriate models for our proposed studies. An important property of mouse models is also that there are suitable inbred GA mice that

have, for example, human forms of key receptors that increase their predictive value as models for humans.

The methods that we and others for the planned experiments are designed to minimise suffering and distress to the mice whilst at the same time, result in useful experimental data. For example, the effects of the engineered proteins in mice with relatively small and non-metastatic tumours that grow subcutaneously will be assessed and this is expected to result in mild effects in most mice, with moderate in about 5% of mice. Humane endpoints in tumour bearing mice are designed to limit suffering (e.g. mice will be culled if and when the tumours reach a moderate size, or mice start to show signs of ill-health such as hunching).

The engineered proteins will be delivered into mice with experimental autoimmune encephalomyelitis (EAE) of mild-moderate severity that can subsequently progress to severe EAE. Two slightly different models of EAE will be used since they give different experimental information. We have chosen to use EAE models instead of other autoimmune disease models since many of these autoimmune disease models cause considerable suffering (e.g. arthritis models, skin blistering disease such as pemphigus vulgaris) and do not have the reagents available to assess immunological responses. If we elected to use these other models, substantial optimisation of new reagents would therefore need to be carried out which would necessitate the use of large numbers of mice with possible failure to generate the reagents/assays combined with needless suffering of the mice. We have spent a considerable amount of time developing and implementing EAE protocols so that the disease activity is at the lowest level necessary to achieve our experimental goals. However, it is possible that quadriplegia could occur in a low percentage of the mice (1-5%). To avoid this, an enhanced monitoring regime will be implemented (more than 3 times daily where appropriate). If any mouse begins to show signs of neurological problems with a forelimb, it will be immediately killed.

Prior to carrying out experiments in mice with EAE, we will perform experiments in mice that have ongoing immune responses (immunised, and in some cases, with passively transferred antibodies) to identify the engineered proteins that are the most effective in reducing inflammatory responses following delivery. The most effective proteins (estimated to be about 50% of the test engineered proteins) will be used in subsequent EAE experiments. This approach represents a significant refinement since it results in the use of reduced numbers of mice in EAE experiments that are associated with higher incidence of adverse effects compared with the effects in mice that are immunised under conditions that induce inflammatory responses but not disease.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

We need to use adult mice for these experiments since we need to assess the distribution of antibody/antigen/Fc-antigen fusions at the whole body level over a period of up to two weeks and this cannot be assessed in mice that are terminally anaesthetised. Similar constraints apply when using mice as models for cancer and autoimmune disease, except the time frames of the experiments are longer.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Mice are broadly taken to be instructive models for the analysis of immune responses, with the goal of modifying these immune responses to treat human diseases such as autoimmunity and cancer. A further advantage of using mice is the availability of genetically altered strains. To ensure high welfare standards, good animal husbandry, including environmental enrichment, is employed. Generally, for this project licence application we expect that the severity of the procedures will be mild for the majority of mice/procedures, but the procedures are categorised as severe for a low percentage of total mice. For all procedures, the mice will be carefully monitored and if adverse events are observed, steps will be taken to alleviate them or the affected mice will be humanely killed.

Animals will be maintained by qualified technicians who have familiarity with many different disease models. Mice will be monitored daily by the animal house technician(s) and the researcher(s) carrying out the experiments. If one or more animals are anticipated to be close to reaching a humane endpoint (the humane endpoints are well defined for the disease models that we plan to use), they will be monitored more closely. If the animal shows signs of approaching a humane endpoint, the animal will either be killed immediately (with a humane method) or the corresponding researcher will be informed that the animal needs to be killed immediately (using a humane method). If mice show signs of distress for which a cause cannot be identified, onsite veterinary assistance will be sought.

Death will not be used as an acceptable endpoint in any of our studies. The tumour and autoimmune disease models that we plan to use are well characterised and humane endpoints have been identified. In many cases, we can assess the size of the tumour and use a limit of this as an endpoint. Occasional ulceration of tumours can also occur, usually in outlier mice, and if observed such outlier mice will be humanely culled.

In the autoimmune disease model that we plan to use, mice may have paralysis in their hind limbs and typically recover from this within several days (65% mice have severe or complete hind limb paralysis).
Such mice will have damp food placed on their cage floor and their weight will be carefully monitored. They will also be housed in groups so that they can huddle to maintain body warmth.

During all experiments, other measures of distress for the mice will be assessed, such as hunched posture, abnormal feeding and drinking, or poor condition that can be detected using other methods such as alterations in facial expression. We have onsite veterinary assistance to provide advice if and when necessary.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

https://www.nc3rs.org.uk

How will you ensure you continue to use the most refined methods during the lifetime of this project?

The personnel involved in this project will regularly review the literature and websites (e.g. https://www.nc3rs.org.uk) for refinements etc.

Explain the choice of species and the related life stages

Mice will be used in these studies since they are the most suitable model. They have been used extensively in studies to model the immune response in humans. In addition, mouse models are accepted as instructive for human diseases such as autoimmunity or cancer. We will use adult mice (6- 12 weeks old) throughout our studies since these are of an appropriate age for the disease models that we plan to use.

A retrospective assessment of refinement will be due by 25 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

3. Aquatic regulatory toxicity testing

Project duration

5 years 0 months

Project purpose

- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)
- Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

No answer provided

Animal types	Life stages
Carp	juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.
What is the aim of this project?

To assess the toxicity and pathogenicity of microbial pesticides to fish. These data are required by European Member State regulatory authorities for the registration of new plant protection products under Regulation (EC) No 1107/2009.

Retrospective assessment

Published: 25 July 2023

Is there a plan for this work to continue under another licence?

No

Did the project achieve its aims and if not, why not?

6 studies were run under this licence to obtain information on the toxicity and pathogenicity of microbial pesticides on fish for regulatory submissions. Trout (Oncorhynchus mykiss) were used in all studies, as they are the standard species accepted by regulators. The studies ran successfully and provided the required information on toxicity and behavioural effects.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The assessment of aquatic ecotoxicity, which includes effects on algae, aquatic plants, invertebrates and fish, is an essential component of the risk assessment process for plant protection products. This testing is mandated and codified by regulatory authorities in most developed countries, including the EU and the US.

What outputs do you think you will see at the end of this project?

This project will result in the generation of the mandatory regulatory data that are essential for the environmental safety assessment of new plant protection products. This assessment is a critical aspect of the registration process for such products in Europe and the US in order to identify and mitigate any negative environmental impacts that may arise from the use of the products.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The benefit of the outputs from the project will be the authorisation of novel products with modes of action that present greatly reduced risks to the environment compared to more conventional materials.

Where these data are required by regulatory authorities as part of product renewal procedures the benefits will be realised over short timescales of a few months; where they are required as part of regulatory submissions for the approval of new active substances the benefits will only be realised once the evaluation process has completed. This can range from 18 months in the case of the US to up to 5 years in the case of Europe.

How will you maximise the outputs of your work?

Whilst the environmental risk assessment and the information on which it is based is made public by the European Food Safety Authority during the public consultation phase of new active substance evaluation, as a contract research organisation the work we undertake on behalf of our clients is commercially confidential and can thus not be published or disseminated by us.

Species and numbers of animals expected to be used

- Other fish: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Fish are exposed to varying concentrations of experimental products in their aquarium water. Fish are observed closely for the duration of the study to determine any adverse clinical signs arising from exposure.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Various clinical signs may be observed throughout the tests, depending on the nature of the test material. These can range from no observable effects at one extreme to death at the other, with the following observations lying in between: dark pigmentation, slight increase/ decrease in respiration, swimming at tank base/water surface, hyperactivity, lethargy, irregular breathing or occasional gulping, slight or temporary loss in equilibrium, increased mucous secretions, very hyperactive, very lethargic, greatly increased respiration or gulping/ irregular breathing at water surface, loss of equilibrium with violent, erratic movements, and muscle spasms.

Severe reactions to the test material will be identified and may constitute a valid endpoint without the need to prolong the exposure, thus avoiding any potential for unnecessary suffering.

Expected severity categories and the proportion of animals in each category, per species. What are the expected severities and the proportion of animals in each category (per species)?

Due to the nature of these tests the expected severity threshold is set at severe. This can potentially be the case for all animals within each test except for those in the control group. It is always the case that animals are exposed following step wise procedures and observed continuously to avoid any potential for unnecessary suffering.

What will happen to the animals at the end of the study?

- Killed

Retrospective assessment

Published: 25 July 2023

What harms were caused to the animals, how severe were those harms and how many animals were affected?

No harm was caused to any of the fish (Oncorhynchus mykiss) involved. No trout suffered any clinical signs, severe reactions, behaviours or conditions throughout the studies conducted. No fish out of the 213 ordered were returned as suffering, mild or moderate severity behaviours.

All fish were killed humanely using the schedule 1 method at the end of each study. Across the study time periods from 2020 to 2021, a total of 213 rainbow trout were ordered

for study purposes, kept in-house under good husbandry conditions and used on relevant and required toxicology studies.

Husbandry and welfare was our main priority and everything was put in place to reduce stress and harm to the fish during acclimatisation periods and while on study.

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

EU and US regulatory authorities require data from vertebrate test systems, including fish, in order to identify and mitigate any adverse environmental effects that may arise from the use of new plant protection, biocidal, animal health or pharmaceutical products. The evaluation of these studies, all of which are performed in accordance with highly prescriptive internationally recognised guidelines, forms a critical part of the mandatory environmental risk assessment for all new products.

What was your strategy for searching for non-animal alternatives?

Currently there are no accepted non-animal alternatives available for aquatic ecotoxicity testing. However, steps will be taken within each study to ensure that the minimum number of test organisms is used. These steps include the minimising the use of attenuated test item and sterile filtrate control groups where no adverse effects were observed in prior *Daphnia* studies, and sharing controls between concurrent studies wherever possible.

Why were they not suitable?

EU and US regulatory authorities require *in vivo* data from vertebrate test systems in order to identify and mitigate any adverse environmental effects that may arise from the use of new plant protection, biocidal, animal health or pharmaceutical products. *In vitro* alternatives are either unavailable or unsuitable, particularly for microbial test items for which no validated *in vitro* methods exist.
It will be company policy to consult with the DB-ALM, ECVAM, NC3Rs and FRAME websites frequently and research any viable replacements for *in vivo* testing as part of the AWERB agenda.

Retrospective assessment

Published: 25 July 2023

What, if any, non-animal alternatives were used or explored after the project started, how effective were they and are there any lessons worth sharing with others?

Throughout the study time periods where fish were used, no direct replacement technology was available.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

A standard OECD 203 / OCSPP 885.4200 microbial pathogenicity study would require between 20 and 40 fish depending on the requirement for attenuated test item and sterile filtrate groups.

2500 fish is the maximum number the facility is currently able to test for the duration of this project.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Regulatory studies will be conducted in accordance with OECD or OCSPP guidelines, which specify the minimum number of fish required to achieve statistically valid scientific outcomes. The minimum number of fish stated within any required guidelines will be used wherever possible, with any deviations from the guideline documented and scientifically justified within the study data.

Prior to conducting any studies on fish, information on the likely toxicity or possible effects of the test item will be requested or researched. If no such information is available dose ranging tests can be performed prior to the main definitive test.

It is often the case that work involving fish will be part of a larger suite of studies conducted on terrestrial and aquatic invertebrates. When this is the case, and where possible, studies will be conducted on invertebrates first in order to identify approximate toxicity and therefore minimise adverse effects in fish studies.

What other measures apart from good experimental design will you use to minimise numbers?

For chemical test items, the data sharing rules implemented under the REACH (Registration, Evaluation, Authorisation and restriction of Chemicals) legislation will help to reduce unnecessary animal testing. Toxicity data are also publicly available on the ECHA database which may preclude the need for additional testing.

For microbial test items there are limited options for reducing the need for fish testing. Survival in water studies, even if they demonstrate rapid mortality of the test organism, do no preclude the possibility of infection and pathogenicity within a more suitable environment, such as the body of a fish. Furthermore, as many microbial pesticides are entomopathogenic and fish are entomophagous, the effect on fish of consuming infected insect cadavers must be addressed via the consumption of dosed feed. However, as microbial studies are all conducted as limit tests at a single concentration of 1 x 10^6 cfu/ml the animal requirement per study is low.

Retrospective assessment

Published: 25 July 2023

How did you minimise the number of animals used on your project and is there anything others can learn from your experience?

We were limited in the scope of reduction that we could achieve due to the regulatory requirements of the studies. However the minimum number of fish required per replicate tank was used for each study. Studies were designed to use the minimum number of replicate tanks. Multiple studies were run together where possible, which allowed control replicates to be shared thus reducing the total number of fish required.

Legal requirements and regulatory guidelines recommend to use a minimum of 10 fish per tank for a limit study. All our studies were conducted as a limit test design in order to reduce the number of fish used.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

The only animal models that will be used during this project are rainbow trout and carp. Protocols 1 and 2 are designed to provide regulatory information on the acute toxicity, sub-lethal effects and LC_{50} values of the test item, the latter being determined from inferred clinical signs and avoiding death as an endpoint where possible, although mortality may be expected. Dose ranging tests and careful interpretation of clinical signs (and resulting euthanasia) will minimise distress as much as possible.

For acute studies, fish will be added to test systems individually or in small batches. Severe reactions or effects of the test material immediately after exposure will be identified and staff may then justify a valid endpoint without the need to continue exposing fish to the test system. The exact method used to introduce fish to a test system will be outlined in company Standard Operating Procedures and Study Plans (e.g. addition of fish at 1 minute intervals followed by close observation prior to the addition of the next fish).

An internal policy document containing guidance on clinical signs may be used to assist in implementing endpoints, as required. Protocol 1 tests that estimate the LC_{50} should follow the ENV/JM/MONO (2010)17 document as closely as possible. This allows information required for regulatory purposes to be acquired, whilst minimising suffering and reducing animal use. Where possible, this approach will also be used for Protocol 2 tests.

The fish species to be used is carefully considered in relation to the test material. For example, volatile chemicals should be tested on a species which can endure lower oxygen contents and survive static conditions. For chronic studies, the use of robust species which reproduce easily and achieve life stages in a shorter time frame reduces the exposure period.

Environmental enrichment, with an emphasis on minimising stress, will be used where possible whilst holding, culturing and testing fish. This is mutually beneficial, as many observations thought to be related to the test material could result from stress.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

In vivo fish testing is mandated by the regulatory authorities in Europe and the United States, and studies must be conducted in accordance with internationally recognised guidelines that specify the fish species that can be used. There is thus no scope for using less sentient animals.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

By using defined observation strategies we will ensure constant monitoring of all fish whilst under all regulated procedures. Thus, any issues are dealt with in a timely fashion and the appropriate action is taken. All test designs are continuously scrutinised to ensure that only the minimum number of fish are used, and where possible further reduced, such as by reducing animal numbers within control groups or performing limit tests with a reduced number of exposure groups. The protocols defined under this project will adhere to current guidance, with any new or revised guidance being adopted at the earliest opportunity.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

It is a requirement of EU and US regulatory authorities that studies are performed in accordance with current published and ring-tested designs, such as the OECD and OCSPP guidelines. These guidelines specify the number of fish that should be used to determine scientifically valid endpoints. In addition, the ENV/JM/MONO (2010)17 document (Series on Testing and Assessment No. 126: Short Guidance on the Threshold Approach for Acute Fish Toxicity) will be followed as closely as possible in order to determine the information required for regulatory purposes, whilst also minimising animal use. This document details the use of information from acute toxicity testing on non-vertebrates such as *Daphnia* and algae to determine a suitable threshold concentration at which to expose fish, in order to
determine the species most sensitive to a particular test substance. Where possible, this approach will also be used for Protocol 2 tests.

The selection of species is carefully considered in light of the properties and the end use of the test material. The CVMPIVICH/790/03 2004 document discusses how the potential use of the test item aids in species selection for environmental testing. For example, chemicals and biopharmaceuticals intended for use with terrestrial animals should only be tested on freshwater species. Testing of volatile chemicals requires a species which can endure lower oxygen contents and survive static conditions. Chronic studies investigating early life stage development require robust species which reproduce easily and achieve life stages in a shorter time period, thereby reducing exposure periods.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

It will be company policy to consult with the DB-ALM, ECVAM, NC3Rs and FRAME websites frequently and research any viable replacements for *in vivo* testing as part of the AWERB agenda. Any updated strategies and best practice available will be discussed at the AWERB and implemented if viable, and when global regulatory authorities confirm their acceptance of new study protocols.

Explain the choice of species and the related life stages

The animals selected for the project are those required by internationally recognised regulatory guidelines. The species and life stages selected for each test design are naturally robust, with low natural mortality and are widely accepted by regulatory authorities worldwide.

Retrospective assessment

Published: 25 July 2023

With the knowledge you have now, could the choice of animals or models used have been improved at all? How did you minimise harm to animals during the project?

The choice of fish (Oncorhynchus mykiss) was the only viable option available for the regulatory studies we had to carry out. The fish were supplied by a professional local supplier which meant the effects of stress during delivery were kept to a minimum, as the distance to travel during transit was approximately 20 miles.

The welfare of the fish was prioritised in the study design with observations conducted daily to ensure no pain or distressed behaviour was missed. The study area was in a quiet, separate room with limited walk through access providing a stress free environment. The tanks used were of a good size and maximum water volume, with fitted lids to reduce accidental deaths, via jumping.

The minimum number of fish were used per replicate, this also provided a good tank area ratio per fish and improved behaviour (reduced individuals from fighting).

All studies were conducted to the OECD and OCSPP guidelines with relevant acute toxicity testing done on non-vertebrates such as Daphnia and algae beforehand. This is to ensure that the test item to be used has undergone previous testing with indicator species and is unlikely to show toxicity effects in larger species like fish. It is a regulatory requirement to do all three studies as a group for aquatic ecotoxicology and also to try and reduce unnecessary fish studies from being done as a replacement method. For example, if high toxicity was observed in both the algae and daphnia studies then the results would be assessed and discussed as to whether a fish study should occur. Repeated studies would then be conducted with daphnia and algae in order to achieve more information on the effects of the test item at different concentrations. For agricultural products which are of a microbiological, fungal and biopesticide origin or component it is very unlikely that a toxic effect would be seen in larger organisms like fish. In this area of research, the fish studies that were run are for a regulatory requirement to show positive results that the test item is not toxic to fish.

Only acute fish studies were conducted using juvenile fish (O.mykiss) of the recommended weight and length ranges. No early life stage development studies were conducted.

4. Autoimmune diseases of the CNS and their treatments

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The aim of this project is to understand how the insulating material around nerves (myelin sheaths) is destroyed by the autoimmune disease, multiple sclerosis (MS), and then to identify and screen agents that are reparative or control the associated symptoms of this disease, with particular attention to neuronal death, loss of myelin, axonal damage and degeneration.

A retrospective assessment of these aims will be due by 29 December 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these

could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

At present there are no therapies that reverse the debilitating disease symptoms of MS, current treatments are palliative and only provide support to the body's natural defence system (the immune system) to fight the consequences of MS rather than tackle the underlying causes. We wish to identify and test effective therapeutic agents that change the immune system to fight the development of MS and to test agents that will ultimately protect neurons and their associated cells and prevent neuronal death, axonal damage, loss of myelin and eventual loss of nerve function.

What outputs do you think you will see at the end of this project?

The primary outputs of this work will be the information on identifying new therapeutic targets to prevent the development of MS or promote repair after MS. As well as identifying new targets, we will also specifically target an already identified therapeutic molecule called AMIGO3 that can promote myelin repair in MS.

We will establish if targeting the intestinal lymphatic system is a viable way to reach concentrations of cannabinoids that are required to treat the clinical signs of MS.

We will also establish if inhibiting DNA damage sensing can promote RGC survival and axon regeneration and hence preserve visual function in models of optic neuritis.

Specific academic outputs will be to publish the findings. This is important for the development of the project but also to provide a knowledge base for other academics working in this field.

Specific product outputs will be to support the development of the intellectual property already filed and allow it to be translated into a commercially viable proposition for the REDACTED of Birmingham.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

In the short term the benefits would be to provide high impact publications and work for the academics involved and in raising the profile of early career researchers. This work will also potentially support an application to the MHRA for a clinical trial of the AMIGO3 or DNA damage antagonist and the intestinal delivery route for cannabinoids.

In the longer term this work has the potential to have an immense impact on the lives of patients with MS. There is currently no treatment that reverses the pathological effects of MS and the functional loss. Our AMIGO3 therapeutic would provide a suitable, clinically viable antibody-based treatment that can potentially reverse the signs of MS. If the intestinal lymphatic system can deliver high amounts of cannabinoids, this will have a huge impact for MS sufferers and provide an alternative treatment for the debilitating symptoms of MS. Our DNA damage inhibitors, specifically one which has been used in Phase 2 clinical trials in cancer, is a potential fast track route to the clinic after we have been able to show efficacy in our optic neuritis models.

A second beneficiary is health care providers, particularly the NHS. Currently around £50 million/year is spent on MS sufferers. If the AMIGO3 antibody/DNA damage inhibitor treatments and the intestinal lymphatic targeting therapies were successful it would relieve this pressure on the NHS significantly reducing this cost burden of treating MS patients.

How will you maximise the outputs of your work?

We will maximise the outputs of the work by collaborating with academics and companies working in this field to maximise the use of the data we obtain. We will rapidly disseminate the outcomes of the tests whether, negative or positive to inform the academic community and support other researchers developing technologies in this area. We are already working with several small and large pharmaceutical enterprises which we will seek to attract after proof-of-principle experiments have been successful. The AMIGO3 antibodies and the DNA damage inhibitors will need to be provided by companies collaborating with us, however, we are currently filing re-purposing patents for use in our disease models and hence there should be no problems with the freedom to publish our results and without restrictions. However, many companies require vetting of each publication prior to submission, but we will ensure this is completed in a timely manner.

Species and numbers of animals expected to be used

- Mice: 1,650
- Rats: 550

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

For the EAE model: animals will undergo subcutaneous injection of myelin antigens emulsified in complete Freund's adjuvant with repeat injections of pertussis toxin at day 0 and 2 to sensitise the immune system. Animals will then be monitored until clinical signs of disease appear (typically between day 17-24). As soon as the clinical score reaches peak of acute disease (score 3 for EAE (hind limb paralysis)

For the optic neuritis model: animals will undergo subcutaneous injection of myelin antigens emulsified in complete Freund's adjuvant with repeat injections of pertussis toxin at day 0 and 2 to sensitise the immune system. Animals will then be monitored until clinical signs of disease appear (typically between day 9-12) and when clinical score of 1 is reached (i.e. limp tail), we will kill the animal and harvest tissues for analysis. Treatments will normally be administered both pre- and post- symptomatically to determine efficacy of therapeutics over a wide range of disease stages.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Induction of disease: During EAE induction phase an initial weight loss of <10% of pre-disease bodyweight observed overnight, then limp tail, ataxia and usually hindlimb paralysis for a period of 2-5 days with further weight loss (even when fed orally by gavage).

During optic neuritis, animals lose 5% of bodyweight prior to disease and then progress through the disease stages as in EAE and can lose a further 5-10% bodyweight. However, we will kill animals with a score 2 (limp tail and impaired righting reflex).

Weight loss is a feature of the disease but animals will be given soft mash in cage when hind limb paralysis occurs. Animals will be checked and weighed daily from day 0 and when in peak disease phase animals will be examined more frequently (not less than twice daily).

Subcutaneous injection of Freund's adjuvant: In less than 20% of animals, granulomas may form at the injection site. However, injection into the flank minimises these adverse effects and animals show no untoward side effects. Ulceration of the injection site is rarely observed (<1% of animals) but heal on their own and are minimised by good ascetic handling. If a granuloma increases to >5mm then animals or if an ulcer does not heal within 3 days, animals will be killed immediately.

Substances administered by injection: Stress due to restraint and transient discomfort from needle insertion is likely in 100% of animals. These are minimised by selection of Appropriate (minimum possible) sized sterile needle and syringe will be used.

Expected severity categories and the proportion of animals in each category, per species. What are the expected severities and the proportion of animals in each category (per species)?

About 60% of animals will be used for EAE experiments and will be expected do develop hind limb paralysis with up to a maximum of 25% bodyweight loss. These are classed as severe. Animals may also receive injections of therapeutic agents or through the use of mini-pumps or slow release devices.

The other 40% of animals will be used for optic neuritis experiments which can be classed as moderate severity since animals are not required to display clinical signs of hind limb paralysis. Animals will be killed when they reach a score of 1 (limp tail). These may also receive therapeutic agents as described above.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 29 December 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Whilst some elements of immunity, neuroregeneration and repair can be modelled in cell culture, the complex, clinical picture and interaction of the immune and nervous systems cannot all be currently modelled in cell-culture or computer-based models. The use of live animals is therefore unavoidable and essential for drug discovery and to demonstrate the activity of drugs in a situation relevant to human disease. Neurons are not present outside the animal kingdom and so an animal is required. Only mammals have a sufficiently developed immune-system to readily compare to humans, and rodents are the animals of lowest neurophysiological-sensitivity required to achieve the scientific aims.

It is not ethical to conduct experiments on humans in multiple sclerosis, especially where those experiments require the removal of parts of the immune or nervous system for ex-vivo investigations. Therefore, there is no feasible alternative that would entirely replace the use of a living animal that would allow the objectives to be met. However, we will use in vitro and ex vivo work prior to or in parallel with animal studies.

What was your strategy for searching for non-animal alternatives?

There are currently no alternatives to animal work for this disease. For example, no cell culture-based models exist that encompass all of the aspects of disease for any of the models described in this project. However, individual aspects will be modelled in vitro and ex vivo. For example, neuroprotective therapies are usually screened in a retinal cell culture model prior to in vivo use. So far we have successfully translated many neuroprotective therapies from this culture-based model to in vivo studies. Also, oligodendrocyte precursor cell differentiation has been studied in vitro. We have also used postnatal rat pups to investigate myelination.

Why were they not suitable?

The fundamental reason why the use of animals is required is to understand these processes that at present no in vitro methods can model the complexities of the systems involved in this disease. It is difficult to use primary cells to culture all of the different types of cells since they require different growth mediums and factors for survival. Indeed, the reason why many new drugs fail between cell culture and in vivo studies is in the inability to full recapitulate the in vivo environment. Technologies are being developed to address this gap, including the development of 3D cultures. However, none of these model systems are yet able to phenocopy the integration and interplay between the numerous cell types that constitute MS/EAE/Optic neuritis as it is a full interplay of the immune and central nervous systems. Modelling MS in rodent is thus still required to fully model disease progression and identify novel therapeutic avenues.

A retrospective assessment of replacement will be due by 29 December 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Sample sizes have been calculated using the NC3Rs Experimental Design Assistant (EDA). In general, setting a significance level of 5%, a power of 80%, and a least biologically significant difference between groups of 30% (i.e. reduction in clinical disease severity after AMIGO3 antibody treatment or protection of 30% of RGC from death), returned group sizes of 8-10 animals/treatment group.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

We used the NC3Rs EDA system to calculate animal numbers to be used from this project. We used sd values from several of our own and other's published data to facilitate power calculations and reduce animal usage.

What other measures apart from good experimental design will you use to minimise numbers?

We will seek to refine protocols, such as the development of novel quantitative outcome measure that will facilitate “reduction”. Experiments will be planned so they can be published in accordance with the NC3R’s ARRIVE guidelines.

As part of good laboratory practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material; and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and the treatment differences that are to be estimated). We will make appropriate arrangements to randomly assign animals to experimental groups and blind studies.

We will also use the $MOG^{TCR}xThy1^{cfp}$ mouse that has a fluorescent label on retinal neurons so that we will be able to monitor neuronal death in real-time without the need to kill animals.

A retrospective assessment of reduction will be due by 29 December 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Multiple sclerosis (MS) exhibits many pathological processes including relapsing and secondary progressive disease. We aim to use the mouse/rat to model these processes. All EAE models in rodents and primates develop autoimmune mediated disease of the CNS, but few exhibit reproducible relapsing disease and progression. Our chief mouse/rat model will be to examine EAE in Biozzi ABH mice/Lewis rats that have proved a reliable drug-screening tool that is far superior to most EAE models due to its reproducibility and the slow accumulation of disability, which limits endpoints being reached. However, to examine CNS autoimmunity we will also examine other mouse/rat strains that exhibit the most robust response to the defined antigen. Because the autoantigens in ABH mice are so hydrophobic and are not amenable to in vitro use, in some instances we will use other strains where autoantigens are more amenable for in vitro use (e.g. C57BL/6 or SJL mice) or where gene deletions have been generated other strains of mice will need to be investigated, e.g. C57BL/6 mice. Disease will typically be induced by subcutaneous injection of myelin antigens dissolved in complete Freund's adjuvant into the flank. This is very well tolerated and induces disease with high reproducibility, limiting the need for large group sizes and many repetitions of experiments. Although much is known about how to best use animal models to study autoimmunity, few studies have investigated use of EAE models to monitor neuroprotection, repair and symptom control. Using induced-relapses to synchronise relapse to detect neurodegeneration compared with treatment of spontaneous disease, requires animals to be in procedure for less time and requires fewer animals to detect effects. We aim to continue to refine these models and as we apply additional outcome measures such as rotarod analysis, we increase power to detect drug effects and enhance the utility of the models. Through the use of a reproducible system and define endpoints for each objective, we can limit the time in procedure and as a result the suffering that the animal will accumulate as a product of autoimmune attack of itself.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

We cannot use non-mammalian species for this work, as mammals cannot recover from attacks of multiple sclerosis. For example, we cannot use zebrafish because they are able to regenerate their CNS spontaneously. In mice there is established and reliable transgene technology, and established models of MS. There are a large number of genetically modified mutants available and there is extensive amount of work that has already been performed and published using mouse and rat models of MS.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

All therapeutic agents are evaluated and optimised in vitro prior to in vivo application. We keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are done on harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug delivery strategies. Small numbers of animals (i.e. 10 mice) are used in these models to maximise the effectiveness of our post-disease care. These refinement steps significantly reduce animal usage and severity.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment.

Experiments will be conducted in accordance with the guidelines published by the Laboratory Animal Science Association (LASA).

The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We will stay informed by advances in the 3Rs through attendance of seminars and conferences, as well as discussions with the NVS, NIO and NACWOs.

We will review each experiment on completion to determine any refinements that can be applied to future experiments.

Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use.

We will also stay up to date with guidance published by FELASA on the most refined experimental methods.

Explain the choice of species and the related life stages

No single experimental model reproduces all of the aspects of human MS, but generally mice and rats are both used in this application to investigate individual aspects related to disease pathogenesis. For example, mouse models of EAE commonly display secondary demyelinated areas, in which both the axon and myelin undergo degeneration, with myelin degeneration being a secondary phenomenon to the death of the axon. In contrast, rats commonly display primary demyelinated areas, in which myelin damage occurs without primary changes in the axon. In human MS however, both primary and secondary demyelination is present with a prevalence of primary demyelinated areas in the brain and spinal cord (Lassmann and Bradl, 2017, Acta Neuropathol 133: 223-244). Hence, both rats and mice are required to model these nuances.

In addition, the mouse lends itself easily to the creation of knockout models and hence offer the advantage of investigating the impact of a particular gene to MS disease progression. Moreover, both adult mice and rats are also chosen since all of the

preliminary work and project tools have used these animals and hence is required for timely progress to be made.

A retrospective assessment of refinement will be due by 29 December 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

5. Brain plasticity with experience and recovery

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Plasticity, Learning, Stroke, Exercise, Experience

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

We aim to understand how experience can change brain structure and function. We will measure brain changes using brain scans (MRI), brain recordings and post-mortem microscopic measures. We will use the approaches to answer the following questions:

How does the brain change with experience?

The types of experience we will study include learning (e.g., learning a new movement skill), physical exercise, sleep and environmental enrichment. In addition, we will mimic changes in experience by directly stimulating brain activity using electrical or optical stimulation. We will test how these types of experience alter brain structure and function.

In some experiments we will test the role of specific genes in these brain changes by modulating genes in rodents. In some experiments we will test whether drugs can modulate the brain changes. These studies allow us to find out which biological mechanisms are responsible for the changes we observe.

The brain's capacity to change varies through the lifespan. Most of our experiments will be in juvenile and adult rodents but we will also study changes in early brain development, where the brain is particularly susceptible to change. We will not study ageing.

How does the brain change with recovery from injury?

Following brain damage, such as stroke, the undamaged parts of the brain adapt to enable some degree of recovery to take place. We will create an experimental stroke in rodents by applying a drug that constricts blood vessels in the brain and mimics some of the effects of stroke. We will measure changes in brain structure and function as rodents recover after this damage.

In some experiments we will test the role of specific genes in these brain changes by modulating genes in rodents. In some experiments we will test whether drugs, brain stimulation, or behavioural training can modulate the brain changes or improve recovery. These studies allow us to find out which biological mechanisms are responsible for the changes we observe and allow us to identify potential therapeutic approaches using combinations of drugs, brain stimuation or training. In our studies in humans we can test these approaches, where feasible, in people who have had a stroke.

What are we measuring with MR scans?

Brain scans are commonly used to study brain change in humans, but it is not possible to know which microscopic changes are causing any observed change in brain scan measures. By acquiring brain scan data, brain recordings, and microscopic information in the same animals, our project will allow us to test which microscopic changes underlie observed changes in brain scans. The results of this project will be used to inform studies in human volunteers. Specifically, the animal work will allow us to develop human MRI methods that provide information on microscopic changes that underlie the changes we can detect using MRI scans.

A retrospective assessment of these aims will be due by 17 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The major expected benefit is new scientific knowledge. We aim to answer these questions:

How does the brain change with experience?

In years 1-3 we will provide evidence on how learning, 'lifestyle' (eg sleep, physical activity), and stimulation alters brain structure and function. In years 3-5 we will find out how genetic and pharmacological manipulations modulate these effects and how these effects vary over the lifespan.

How does the brain change with recovery from injury?

In years 1-2 we will provide evidence on how the brain changes with recovery from damage. In years 3- 5 we will test how this can be modulated by behavioural or drug manipulations. Answering these questions has potential clinical benefits as these discoveries can be translated into human trial after stroke. Some approaches (e.g., behavioural training or drugs that are already safely used in humans) could be translated into human trials within 5 years. Others (e.g. types of brain stimulation) would require time to translate into equivalent approaches for use in humans and would therefore develop over the next 10 years.

What is the biological basis of brain MRI signals?

Brain scans are commonly used to study people with brain disorders. However, brain scans typically give us indirect measures of microscopic features of the brain tissue. For example, they might tell us about water content, rather than the number of cells in a brain area. Our work will help to interpret brain scan information in terms of the underlying biology. During the 5 years of the project we will develop new methods for MRI scanning and analysis that will allow us to derive measures that relate more closely to the underlying biology.

The underlying biology is relevant to understanding clinically important questions such as how people recover after stroke. We therefore hope that the work will help to identify measures from brain scans that could be useful clinically, for example in guiding rehabilitation after stroke.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We expect to use up to 4250 rats and up to 7500 mice over 5 years.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

Behavioural testing

Some of the behavioural tests that we use require rodents to work for food rewards. For these test to work well the animals need to be hungry. We therefore limit their access to food prior to testing. This risks weight loss. We monitor weight carefully and supplement food if needed. If a rodents' weight remains low for a sustained period the the rodent is killed.

Some tests involve brief periods of swimming. This risks hypothermia and stress. We control water temperature and limit the time rodents are in the tank to minimise this. Each swimming period is limited to a maximum of 2 minutes for rats and 90 seconds for mice and animals are dried or provided with a heat source on being removed from the water.

These steps normally minimise any adverse effects but we monitor recovery and if signs of hypothermia or stress persist for more than 2 hours after the test then the rodent is killed.

Some tests involve electric shocks being delivered to the rodents feet in order to test how well the animal can learn to avoid the shock. It can take a few days for the animal to learn how to avoid the shock (for example by pressing a particular lever) and during that initial learning period the animal experiences shocks that it cannot avoid.

Substance administration

Some rodents will recieve substances such as drugs. We use appropriate delivery methods to minimise this.

If injections are repeated this risks pain, distress and increased sensitivity. To minimise these we limit number and frequency and vary injection site. Animals exhibiting prolonged signs of suffering would be killed.

Gavage can lead to weight loss. We monitor weight carefully and supplement food if needed. If a rodents' weight remains low for a sustained period the the rodent is killed. Use of minipumps very occasionally leads to inflammation around the pump. We will monitor carefully and intervene as required. Any rodent with persistent weight loss and malaise would be killed.

Some specific substances we use can cause adverse effects which will be carefully monitored. If rodents experience prolonged weight loss, persistent malaise, or prolonged unexpected change in behaviour they would be killed.

Surgery, recording and intracranial substance administration

Any surgery risks infection and pain. Analgesics and appropriate surgical procedures will be used to minimise this risk. Any rodent not fully recovered within 24 hours will be killed.

Brain surgery and/or use of implants carries small risks of haemorrhage, swelling, or infection during or after surgery. We will monitor closely and treat as required. If rodents have persistent weight loss or if interventions are not effective then they will be killed.

Sensory/optical/electrical stimulation and pharmacogenetic manipulations

These carry a small risk of seizures. Rodents will be monitored and stimulation or dose adjusted if needed and anticonvulsants given if needed. If seizure activity persists the rodent will be killed.

MRI scanning

Most MRI scans will be carired out under general anesthesia which carries small risk of anesthetic death. This risk will be minimised through monitoring and appropriate surgical procedures. If a rodent does not recover as expected and does not respond to interventions then it will be killed.

Some MRI scans require a contrast agent to be injected prior to the scan. This risks pain which will be minimised via analgesics and appropriate dosing. If a rodent shows persistent weight loss or does not respond to remedial interventions it will be killed.

Some MRI scans will be conducted with the rodent awake and performing a task. Typically, we would use liquid rewards to motivate the rodent to perform the task. This requires the rodent's access to water to be restricted prior to the test. This risk dehydration. We will use minimal water restriction required to motivate performance, will monitor the animals and will provide free access to water if a rodent shows persistent signs of dehydration beyond that needed for the task. If the rodent does not put on weight and recover in response then it would be killed.

Blood sampling

Occasionally we need to take a blood sample which risks blood loss. We limit volume taken and limit number and frequency of blood draws. If excessive blood loss cannot be controlled the rodent would be killed.

Stroke model

We will use inject a vasoconstrictor into the brain to mimic some of the effects of stroke. This will be expected to lead to behavioural impairments which will vary depending on the site of the lesion. For example, if we target the motor cortex we expet to see movement problems. Partial recovery is expected but many animals will have permanent deficits as a result of the lesion. We expect animals to recover suffiicently to be able to feed and groom themselves. If rodents experience persistent weight loss or fail to respond to interventions and are unable to look after themselves then they would be killed.

There is a small risk of unexpected cerebral swelling or bleeding. If a rodent shows persistent signs of this it would be killed.

There is a small risk of seizures immediately after surgery which would be treated appropriately. If seizures persist and do not respond to treatment then the rodent would be killed.

The general risks for surgery given above are also relevant here.

Experiments with pups

Some of our experiments aim to study the developing brain and so we will investigate rodents from birth. Studying the first days to weeks after birth is important as this allows us to understand how the brain develops. There is a risk that pups that have been taken away from their mother (e.g. for an experimental procedure to be performed) will be rejected by the dam when returned to the home cage. To minimise this risk we ensure that experimenters handing pups first rub their gloved hands in used nesting material to ensure that the pups continue to smell like the home cage. Also we ensure that pups are active and have returned to their normal colour before being returned to the dam. In our previous experience this approach significantly reduces the risk that the dam will reject the pup.

Use of neuromuscular blockers

Functional MRI (FMRI) scans are very sensitive to movement of the animal being scanned. If the animal moves then data may not be usable. Therefore, to minimise movement we use neuromuscular blockers in some experiments using FMRI. Experimenters using this approach will have been specially trained in the use of neuromuscular blockers. Animals undergoing this procedure will be very carefully monitored and anaesthetic will be delivered along with the neuromuscular blocker.

Enforced fluid and food restriction

Animals that undergo enforced fluid or food restriction may experience weight loss. Any animal who drops below 85% of their lean starting body weight for a period of 24 hours will be given additional food or water. If weight gain does not occur within 72 hours, the rodent will be killed. Any animal dropping below 80% of their lean starting body weight will be killed.

A retrospective assessment of these predicted harms will be due by 17 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

The majority of our research is conducted in human volunteers, including individuals who have had a stroke. We use brain scans to study how the brain changes with learning, experience, or recovery from stroke. However, in order to test how brain structure changes at a microscopic level, and to relate this to brain imaging measures, it is it necessary to do experiments in which brain scans can be taken in animals that can then be killed to perform histological measurements. This is not ethical (or practical) in humans. Computer simulations of the brain actually rely on the information that we will provide and so cannot replace the work that we do.

A retrospective assessment of replacement will be due by 17 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

We seek to reduce the number of animals use through:

MRI scanning

Our primary focus is brain change, which requires us to measure how the brain changes over time. MRI can be used to scan the same live rodent over time. This uses far fewer animals that would be required for methods that are performed post mortem.

MRI also provides measures from across the whole brain at once, whereas many other techniques focus on a single brain region and so if multiple regions are to be studied this requires many more animals.

MRI provides measures of both brain structure and brain funciton which can be easily acquired from the same rodent in a single scanning session. Most other methods either assess funciton (e.g., electrophysiology) or structure (e.g,. histology).

Sensitive behavioural tests
We use very carefully designed and sensitive tests that can pick up subtle changes in behaviour. This reduces the number of animals needed to find a meaningful effect.

Training

Our researchers are highly trained to perform tests accurately and consistently in order that they can produce reliable measurements which again reduces the number of animals needed to detect an effect.

Pilot studies

We first use pilot studies with small numbers of animals to refine interventions, validate measures, trial drug doses so that minimal doses can be used, and pilot surgeries (eg under terminal anesthesia). This means that our approaches are as refined as possible, and are more likely to work, when we embark on full scale studies.

Efficient breeding

We use experienced staff to ensure our breeding strategies are optimal in minimising animal numbers.

Experimental design and statistics

We use appropriate experimental design methods, such as randomisation and blinding, to ensure our conclusions will be robust. We use control groups so that we can rule out alternative explanations for our effects (eg the brain might change over time due to development rather than due to our experimental intervention). We perform power calculations so we can ensure we use enough animals so that our results will be meaningful.

A retrospective assessment of reduction will be due by 17 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We work on rats and mice because they are the lowest vertebrate group which reasonably resembles humans.

We use strict limits on what each animal can experience, limiting the number and duration of test and imposing minimal rest time between tests for example.
Many of our experiments use MRI brain scans. MRI is a non-invasive method that is also used in humans. In most of our experiments rodents will be scanned under general anesthesia and will be carefully monitored to ensure appropriate levels of anesthetic are provided. For some MRI experiments we will scan animals while they are awake and in some studies the animals will peform a behavioural task while they are in the scanner. The animals are extensively trained beforehand so that they gradually get used to the experience of being in the scanner.

Many of our expeirments involve behavioural tests. Where possible we use spontaneous behaviours that the animals would perform naturally, such as exploring a new environment. Some tests involve the animals working to receive a food or water reward. In order for these tests to be useful the animals have to be slighly hungry or thirsty so that they are motivated to work. We carefully monitor the animals to ensure that the right level of diet restriction is used.

Some of our tests involve experiences that can be stressful to the animals, such as swimming or experiencing a brief shock to the foot. These tests are only used when there is no suitable alternative to assess the cognitive process of interest. For swimming tests we limit the amount of time the animal does the test and monitor water temperature to reduce discomfort. For footshocks we limit the amount and frequency of shocks and monitor carefully. Some of our tests involve exercise. Rodents can be made to run on a treadmill using shocks. We will use other methods of encouraging running by using obstacles. If animals are experiencing any aversive tests then we will limit the other tests that they undergo.

Some of our studies involve sleep deprivation. We will achieve this by observing animals and providing novel objects or sounds when they start to sleep.

Where possible, animals will be housed in groups. Some studies require us to house animals individually - for example so that we can monitor the activity, sleep or diet of an individual. Methods are being developed that allow for individual monitoring using technology such as remote tagging. We will aim to use these methods wherever possible.

Operations on the brain are done very carefully using aseptic techniques and in state-of-the-art surgical theatres, and the animals are given pain killers after the operations until they have fully recovered. Soon after most of the operations you would not be able to tell the difference between treated animals and controls as they behave in their home cages. It is only on the sophisticated tests of learning and memory that you can begin to tell them apart.

Some experiments involve mimicing the effects of stroke. There are many different ways in which stroke can be mimiced in animals. We will use a method in which we inject a substance into the brain that contricts blood vessels in the injected area. This typically causes less extensive damage than other approaches and can be more carefully controlled. It allows us to test effect of focal damage. Depending on where the injection is made, some symptoms would be expected. For example, if the injection is made in the motor cortex then we expect to see movement difficulties with limbs on the opposite side of the body. These deficits are expected to partially recover over time but in some animals they will not return to normal. We expect all animals to recover sufficiently well to feed and groom themselves and if they do not then they would be culled. Some experiments involve delivery of a drug. There are different ways to deliver drugs (eg via

injection or in food). We would chose the most appropriate method to minimise discomfort. Sometimes substances are delivered directly into the brain. Methods for doing this will be carefully controlled to minimise adverse effects.

A retrospective assessment of refinement will be due by 17 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

6. Central nervous system nutrient sensing and microglia in development, health, aging and neurodegenerative disease

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

This project will explore how changes in nutrition can impact on brain function throughout life in both health and dementia (neurodegeneration). We will look at how nutrition, and cell sensing of nutrients impacts on cells in the brain, including the brains immune system cells (microglia) and try to understand how these factors may alter brain function.

A retrospective assessment of these aims will be due by 27 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Poor nutrition (either under or over) is a major global concern, affecting over 2 billion adults, and nearly 250 million children. There is a significant body of evidence demonstrating that nutritional availability can have a major impact on brain development and function throughout life, and studies have now identified systems that regulate how our cells respond to different nutrients. Learning more about how nutrition impacts on brain development throughout aging, and the mechanisms that regulate this, will help improve our understanding of the risks of poor nutrition on the brain, which may in turn help us to improve outcomes in people who have suffered from nutritional problems. In addition, nutrition may play a fundamental role in age related neurodegeneration, such as is seen in dementia, and understanding more about the mechanisms that underlie this role will provide new insight into the possible causes of these diseases, and also prospective new routes to therapy development.

What outputs do you think you will see at the end of this project?

Outputs will include new information regarding the impact of nutrition on the brain both in healthy aging and diseases such as Alzheimer's disease. It will lead to several publications that will advance our understanding of how nutrition may be linked to disease and will tell us more about the mechanisms underlying this interaction. It will also improve our understanding of how and why malnutrition during development has a profound and long-lasting impact on brain function as we age, and explore whether any of these impacts can be inherited.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Initial benefits (obtained throughout the course of the project) will be seen by other researchers in the field of aging and neurodegenerative diseases, as they will provide mechanistic information to improve our understanding of how things may go wrong as we age. Longer term (over the course of the project and beyond), data obtained from this project may provide us with new targets against as yet unidentified proteins or genes, to try to develop therapeutics to treat diseases such as Alzheimer's disease, and may also provide us with simple approaches to reducing disease risk, or improving mental capacity as we age, which will have benefits for all.

How will you maximise the outputs of your work?

All data obtained in this work will be widely shared throughout the academic community, with the goal of publishing all findings, positive or negative, in relevant scientific journals. As new findings are obtained, collaborations with both local colleagues and those further afield will be sought out to further develop our research, and if appropriate, relevant pharmaceutical or therapeutic companies will also be approached with the goal of further progressing potential new treatments. In addition, efforts will be made to engage with the local lay community to share any findings of interest, and in cases of specific interest to

patient groups, we will liaise with the appropriate support group to try to disseminate the relevant information widely and accurately.

Species and numbers of animals expected to be used

- Mice: 8500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

The work planned in this project falls into four main categories:

Normal and genetically modified mice will be aged out typically to around 2 years old, and during their life around half of these animals will undergo behavioural tests to look at how well their brain works. These tests will include tests of memory, and tests for mobility. In most cases, mice will be tested 6 times in their lifetime. They may also undergo an MRI scan up to 6 times in their life.

Normal and genetically modified mice will be placed onto an altered diet - either increased fat/cholesterol (12 months max), or reduced calorie/fat/protein (16 weeks max). Around half of these animals will then be placed on another altered diet for a further 16 weeks/12 months. These animals will be regularly weighed, and aged out to typically 2 years, and around half of them will undergo tests of memory and mobility function, as well as MRI scans, typically a maximum of 6 times throughout their life.

Normal and genetically modified mice will receive a dietary supplement or drug related to nutrient control such as statins, usually for their entire lifetime (typically 2 years). Around half of these animals will undergo memory and mobility tests and MRI scans throughout their lifetime (typically 6 sessions for each), with half of these also being placed onto an altered diet, as described above.

Healthy normal female mice will be placed onto a reduced nutrient diet for 3 weeks before being used as breeders. These mothers will be maintained on this low nutrition until their pups are weaned. Pups will continue to be maintained on a low nutrient diet until they reach 6 weeks old, at which point, they will be returned to normal food. These pups will then typically be aged out up to a maximum of 2 years old, and around half of these animals will undergo memory and mobility tests and MRI scans throughout their lifetime (typically 6 sessions for each for a 2 year old mouse).

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Animals on this project that are maintained out to old age may develop various age-related issues that are common to mice, such as benign tumours or hair loss, and in some cases arthritis or sore patches on the skin. If these effects are only mild, and cause minimal

distress, the animal may be maintained for several months, however, if any of these issues are thought to cause significant pain, discomfort or distress, the animal will be culled.

Animals modelling dementia may have learning and memory problems, that progress throughout their life. However, these are not expected to have any major welfare issues for the affected animals, and animals will be kept for a maximum of two and a half years (usually no more than two) as these memory problems develop.

In a small number of cases, where we are looking at models of motor neurone disease, animals may develop problems with mobility, and a mild tremor. This is not expected to be accompanied by any pain, and in most cases, will not affect the mouse's ability to move around their home cage. These animals be kept for a maximum of two years as these mobility problems develop. In rare cases, animals may experience difficulties moving around their home cage, and this impact will not exceed a maximum 2 week duration. This more severe mobility issue may also be accompanied by weight loss, which again is not anticipated to last longer than 2 weeks, and in most cases, will not exceed 1 week.

Animals on an altered diet may experience weight gain or weight loss. This is not expected to cause anything more than minimal discomfort to the animal, and may last for the lifetime of the animal. Animals receiving a dietary supplement or drug known to alter nutrient availability (e.g. statins) are not expected to experience any adverse effects.

Prospective mothers undergoing malnutrition are expected to show signs of weight loss, and may have reduced litter size, or problems carrying a litter to term. The period of malnourishment for these mothers will not exceed 10 weeks. Pups from these mothers which are also experiencing early life malnourishment may be smaller than usual, and may grow and develop more slowly. The period of malnourishment for these pups will not exceed 6 weeks after birth, and no significant additional long term adverse effects are expected as a result of this malnourishment for the rest of the lifetime of the mouse, which will not exceed 2 years.

Expected severity categories and the proportion of animals in each category, per species. What are the expected severities and the proportion of animals in each category (per species)?

Most of the experimental work planned in this project is not expected to result in more than mild to moderate impacts on the animals to be used. Most work from the first 3 categories described above is expected to result only in mild and/or transient symptoms that are not expected to result in more than minimal discomfort. In some cases, animals may experience some level of motor impairment, or weight loss/gain that results in some difficulties negotiating their home environment. However, none of these symptoms are expected to result in any pain, or lead to any long-term significant distress for the animal. Similarly, many animals on this project will be kept to old age, and these may experience a number of issues related to aging, but this will not exceed the mild to moderate symptoms that aging humans experience. This work accounts for approximately 92.5% of the experimental animals to be used under this licence.

The malnourishment of breeding females and their newborn offspring is anticipated to have a more significant impact on the mice to be studied, and may result in prolonged sensations of hunger lasting several weeks, that leads to a more significant level of short to mid-term discomfort. This work accounts for the remaining 7.5% of experimental animals to be used on this project.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 27 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

This project is interested in the interaction between nutrition and brain function, which requires us to be able to study the interaction between food intake and the brain. To be able to fully understand how different nutrition can impact on brain function, we need to be able to explore what happens in response to different long term diets, which can only be readily achieved in living animals. Most of the work in this project is focused on how nutritional changes can affect our brains as we age, including understanding more about whether long term diet can contribute to diseases such as Alzheimer's disease. This requires us to be able to study the brain throughout aging. Both the complex circuitry of the brain and the process of aging are challenging to model outside of a living animal, hence using animals is the only way to address these questions. Finally, we are also interested in understanding how any brain changes we see may relate to changes in behaviours such as learning, and these characteristics can only be measured in a living animal.

What was your strategy for searching for non-animal alternatives?

Various cells, including stem cells (grown in a dish).

Brain slice cultures (thin slices of brain that are grown in a dish).

Why were they not suitable?

Where possible, suitable cell or brain cultures will be used to address basic questions about cell function. Basic cell and stem cell cultures can provide information regarding what happens within a single cell, while brain slice cultures can provide information about local cell networks. However, neither of these options can fully mimic the impact of interactions between the millions of cells within the brain, and also how communication with, and the function of other parts of the body can impact on the brain. In addition, neither cells nor brain slices can give us any information on the practical behavioural consequences of any changes we see.

A retrospective assessment of replacement will be due by 27 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers to be used on this project have been estimated based on total numbers planned for the various different experiments to be conducted under the remit of this licence. For initial studies where basic information regarding expected outcomes for disease model or control mice is already available, power analyses have been conducted based on the expected effect size and variability, and total animal numbers per group have been assigned based on these analyses. For small scale pilot studies, where effect size and/or variability are not known, numbers will be kept small, and based on prior experience of similar studies to give the minimum number of animals expected to be needed to generate solid pilot data for further study. In the case of follow-up studies for this work, current number estimates are based on those typically required for similar experiments, but specific power analyses will be conducted for each experiment to confirm optimal numbers before the experiment begins.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

During the design phase of each experiment, where information was available, appropriate searches were done on each mouse line of interest to ensure we had access to all relevant information regarding expected outcomes in these animals, and this knowledge, combined with more general knowledge on the likely response of an animal to the specific modulations being tested was combined to help drive the power analyses that informed our final animal number choice. Extra consideration was made for a number of variables, including sex, age and background strain. The statistical approaches to be used at the end of the study formed a major part of the experimental design process, ensuring that all experimental approaches are robust.

The experimental design was also discussed with other researchers familiar with these kinds of studies, to further validate the design, and to ensure that we had included adequate control groups to each experiment, such that all data obtained will be valid.

What other measures apart from good experimental design will you use to minimise numbers?

As far as possible, all experiments will be conducted longitudinally, to allow us to maximise the data obtained from each animal, and where possible, multiple data types will be obtained from the same animal (e.g. behaviour and imaging). For tissue harvest, all organs of interest will be harvested from each animal, and experiments will be designed to allow multiple follow ups in the tissue from each animal, thus minimising the total number of animals required.

In many cases, small scale pilot studies will be conducted prior to each full scale study, with follow up analyses to ensure both that the objective is a valid one, and also that experimental design can be optimised to use the minimal number of required animals. This will ensure that unnecessary or poorly powered large scale experiments do not take place.

As far as possible, breeding strategies will be designed so that all animals from a mating are used for an experiment, and for general maintenance of a line, animal breeding will be monitored and controlled to ensure that we obtain sufficient mice to maintain the line, while minimizing the birth of mice that are not required.

A retrospective assessment of reduction will be due by 27 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

All the work planned in this project will use mice. These will include healthy control animals, and also models of diseases such as Alzheimer's disease and motor neurone disease. In most cases, these models will not induce any significant suffering to the animal, as they do not impact on the ability of the mouse to feed, drink and move freely about their cage. In the case of models of motor neurone disease, the animals may develop some problems with mobility. This is a known feature of motor neurone disease, and cannot be fully avoided. However, the models used in this project will either be slow progressing models, that do not develop major paralysis, and can hence still readily access their food and water, and move about their cage, or they will be animals bred only to provide cell and tissue for culture at a point prior to the onset of any obvious disease. These models have been selected because they provide a good model for the disease, while minimising the suffering of the animals.

A number of studies in this project will look at the effect of altering nutrition on brain function throughout aging. The dietary changes to be used have all been previously reported, and are not expected to cause any major long term problems for the mice, aside from some mild changes in weight. These diets are all designed to reflect aspects of human diets, and have been selected as they combine a realistic reflection of human nutritional variation with minimal long term welfare implications for the mice.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

This study is interested in the long term impacts of nutrition on the brain, specifically on the cerebral cortex, a brain region that is only found in mammals. Where possible, embryonic or newly born animals will be used to generate specific cell or brain cultures. However, the long term impact of diet on brain function can only be measured by altering the diet of animals throughout their life, and studying the impact this has. This is a long term process, that cannot be conducted under a terminal anaesthesia, and also requires us to be able to look at the brain function of conscious animals, including measuring factors such as memory. Since only mammals have a cerebral cortex, the region most affected by many diseases such as Alzheimer's disease, the use of conscious adult mice is the only way to address the majority of questions posed in this licence.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Animals undergoing any kind of nutritional variation will be regularly monitored (a minimum of weekly, and increasing on demand), including being weighed, to ensure that no unexpected welfare issues arise unchecked, and if any dietary modifications are found to cause unexpected distress, they will immediately be modified or terminated to ensure we minimise such welfare costs.

Any animals undergoing regular handling as a part of the experiment will be handled regularly prior to the start of the experiment, to ensure that this process does not induce unnecessary anxiety or stress to the mouse. In addition, all mice will receive appropriate training for all behavioural tasks prior to the onset of experiments, both to minimise anxiety and stress, and also to improve outcomes, which should ensure consistent data from all mice, and hence allow the use of the smallest number of tests and animals.

Any animals undergoing dosing will where possible be given the compound in their food or water. Where this is not possible, dosing regimes will be designed to minimise stress and suffering to the animal. Animals will be handled prior to the onset of the experiment, and in the case of oral dosing, a thin flexible plastic tube will be used, and animals will be acclimatized to this prior to the administration of any substance. Animals receiving any compound will be extra closely monitored during the initial treatment phase, to ensure that no unexpected issues arise.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

All work will be conducted following the general principals of the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines developed by the NC3Rs organisation.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

I will regularly check the NC3Rs website for updates, as well as liaising with both our NTCOs and our local NC3Rs regional programme manager. Any relevant changes will be implemented immediately for all new experiments, and consideration will also be given to implementing changes to ongoing experiments, provided any such change is not expected to have an impact on the animals such that it may alter research outcomes.

Explain the choice of species and the related life stages

This project works with mice from gestation out into old age, as we are interested in how nutrition can influence our brain function and development throughout life. The mouse has been selected for these studies, as they have a similar diet to humans, and also possess a brain region called the cerebral cortex, which is only found in mammals. We are especially interested in how nutrition throughout life might alter our risk of developing dementia and similar diseases, and so many of the mice used in this project will be genetically modified to develop aspects of these diseases as they age. Other mice will have genetic alterations to how their brain cells sense different types of nutrients, which will allow us to look at how these nutrient sensing systems behave as we age, and how they may be involved in the development of dementia and related diseases.

A retrospective assessment of refinement will be due by 27 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

7. Cortical and subcortical neuronal interactions during execution and observation of action

Project duration

5 years 0 months

Project purpose

None selected

Key words

No answer provided

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The main aim of this programme is to deepen our fundamental understanding of how the primate brain generates skilful hand movements and how unwanted movements are stopped. I will record activity of individual neurons in the non-human primate (NHP) brain and investigate their behaviour during skilful hand movements. NHPs will be trained to reach and grasp different objects, and to observe a human experimenter grasping the same objects. On some trials, animals will be given a late instruction to stop their movement. Neurons in motor areas of the brain are known to be active during reach and grasp, but it was recently shown that some neurons are also active when animals are not moving themselves but observe the actions of a human experimenter. These neurons are called mirror neurons: their activity during the monkey's own movement is "mirrored" by their activity while it observes a human actor, without moving itself. Observation of the actions of others is central to human social interactions. Although we rarely move ourselves during action observation, much of the brain's motor network is active during action observation. Understanding mirror neuron activity can help us shed light on the fundamental question of how and when movement is generated, and when stopped.

For the first time I will investigate whether mirror neurons are affected by touch. Since touch of an object during one's own movement generates somatosensory feedback that is lacking during action observation, this might provide a key brain mechanism for discriminating execution vs the observation of actions.

Motor areas in the brain that control movements have a special 'hyperdirect' connection to areas of the brain that have been stimulated to treat patients with Parkinsons. This hyperdirect connection may be important for stopping unwanted movements that patients

with Parkinson's suffer.I will, for the first time, investigate the functional role of the neurons in motor cortical areas connected to the STN during execution, observation and stopping of actions.

Different motor areas in the brain are connected to each other, and the strength of these connections can be changed using electrical stimulation. I will investigate how to induce connectivity changes in that enhance motor output for skilful action.

A retrospective assessment of these aims will be due by 28 July 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Skilful hand movements are critical to human culture but are often vulnerable to disease. This project aims to investigate several fundamental neuroscience questions that have potential long-term benefits for human patients suffering from PD (the second most common neurodegenerative disorder, affecting ~ 145,000 people in the UK (www.parkinsons.org.uk) and stroke (~100,000 new strokes in the UK each year (www.strokes.co.uk)). Action observation is a key human characteristic. Although we rarely move ourselves while observing actions, much of the brain's motor network is still active. We also know that observation-related signals clearly survive in paralysed and disabled patients and they could be used to control Brain-Machine Interfaces (BMI). I will be investigating for the first time sensory properties of the mirror neurons. So far, it has not been tested how somatosensory input (touch, proprioception) influences the mirror neuron system. This is important, because incorporation of the somatosensory information into BMIs is essential for the future research and development in this area.

Research in NHPs has helped provide a better understanding of Parkinson's disease (PD), and led directly to the use of DBS of the subthalamic nucleus (STN) to improve motor symptoms of PD. In my research programme, I seek to investigate how cortical motor areas, important for movement, are connected to the STN to better understand mechanisms underlying DBS therapy and how current approaches could be improved.

Understanding of the connections between different motor areas and how these connections contribute to motor function is another major objective of my project licence. I will study how this contribution can be enhanced by using different electrical stimulation protocols known to alter cortical connectivity; such protocols could be implemented for example in rehabilitation after stroke.

In summary, a major, but not the only benefit, of the work outlined in this project license will be for researchers interested in the fundamental properties of the mirror neuron system, movement control, and neuronal interactions between different cortical and subcortical areas during complex behaviour. Results of this work will be widely

disseminated to the scientific community through the publications in scientific journals and presentations on the local, national and international conferences. I have previously published my work in high impact scientific journals. Myself and members of my lab regularly attend international conferences and are invited to present results obtained in his lab in leading international centres of neuroscience.

In addition, the research described in this application will be disseminated to the general public. I am actively involved in communicating the results of my work in the public domain. For example, I was recently interviewed for a BBC3 TV documentary, which explored the need and significance of NHP neuroscience research.

Understanding of basic mechanisms of the mirror neuron system, the functional role of the cortical neurons projecting to the STN, and possible ways to enhance connectivity between motor areas, might have medium and long-term benefits for clinicians, clinical practices and ultimately for human patients.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

8 non-human primates (macaques) over 5 years

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

The procedure involves a number of stages for preparing non-human primates (NHPs) for long-term recording of single neuron activity in the awake state. This includes a number of separate and well- spaced implantation surgeries under deep general anaesthesia. These are carried out under full aseptic conditions and involve a full regime of pre- and post-operative analgesia. All anaesthetic procedures are carried out by qualified veterinary surgeon and all surgeries are performed under his/her supervision. Training and recording sessions involve head and body restraint while recordings are taken from multiple microelectrodes advanced into the brain through small, protected openings in the skull made during surgery. Neuronal activity is recorded while the NHP performs its trained task. Recordings are usually taken from pairs of cortical sites. During the course of these studies, which typically last for 2-3 years, both cerebral hemispheres are investigated.

All animals are pair-housed and are provided with natural light, an enriched environment and have large home cages, exercise pens and forage areas. They interact regularly during the day with investigators. The animal’s health is being constantly monitored throughout the procedure, this includes at least weekly weighing and regular blood tests. The NVS is consulted in case of any behavioural or health problems. Possible solutions to potential problems will be discussed with the NVS, NACWO and HOI when appropriate and if problems could not be adequately resolved, the animal will be terminated. We have had no instances of this in the last twenty-five years of NHP research in this laboratory.

The prospective level of severity is severe. The retrospective (actual) severity level for all animals on REDACTED was assessed as moderate. At the end of this procedure, the NHPs are humanly killed by an overdose of anaesthesia.

A retrospective assessment of these predicted harms will be due by 28 July 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

This research programme aims to widen and deepen our understanding of fundamental questions about how skilful hand movements are generated and how unwanted movements are stopped. It therefore requires studying those parts of the brain that are essential for motor control. Because the macaque monkey and human motor systems are very similar in both structure and function, the macaque model is the best one to use to address the questions posed in this programme of work. Neither New World marmosets nor non-primate animal models have a motor system similar to humans,
and therefore these studies could not be carried out in such species. In addition, there are few substantiated reports of mirror neurons in species other than macaques and humans.

To understand the human mirror neuron system and its role in suppression of unwanted movements, we need some invasive work that will allow us to interpret correctly and to calibrate the results of human non-invasive studies. It is not yet possible to sample activity of single neurons in the healthy human brain, and recordings from small populations of such neurons in a non-human primate model are essential for our understanding and interpretation of non-invasive methods such as functional MRI, near-infrared spectroscopy (NIRs) and transcranial magnetic stimulation (TMS).

Data produced in the experiments described in this license are an extremely valuable source for theoretical modelling studies. Existing models can be tested and verified and new models can be created to inform future research and mechanistic understanding of the brain processes underlying behaviour under investigation and eventually for replacement of some aspects of the experimental research in animals.

A retrospective assessment of replacement will be due by 28 July 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

We use advanced experimental techniques which allow us to record more data simultaneously from different brain areas in a shorter time from a single subject. This directly leads to smaller number of animals being needed before enough data has been collected to allow thorough statistical testing of the scientific hypotheses.

A retrospective assessment of reduction will be due by 28 July 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The use of a non-human primate model is essential for this project. Mirror neurons were first discovered in NHPs. The macaque's motor system, including its mirror neuron component, closely resembles that of the human. Rodent models could not be used for this research due to substantial anatomical differences and their inability to perform the complex tasks required to achieve the aims of the project.

All of the techniques used in this laboratory have been refined to make it possible to carry out long-term neuroscience studies in young macaques, and we have had sustained success in terms of scientific productivity combined with optimal welfare.

Single-neuron neurophysiology in combination with stimulation techniques is the chosen method because it provides unparalleled inferential power to determine the causal role of neuronal activity in directly influencing behaviour. Simultaneous recordings of single neurons and local field potentials at different cortical and subcortical locations offers multiple advantages and refinements over traditional single neuron neurophysiology, including the ability to determine how different brain regions functionally interact to guide behaviour. In addition, such techniques produce substantially more data with fewer animals and fewer recording sessions than conventional single neuron neurophysiology.

We use high-resolution magnetic-resonance imaging (MRI) to guide and confirm targeting of brain areas including deep structures. This is an essential refinement since it reduces the number of surgical procedures and increases the accuracy of recording and stimulating electrode placement.

In combination with MRI, we extensively use 3D printing for development of implant prototypes. This allows us to design more complicated implants to reduce time of a surgical procedures needed to prepare animals for neuronal recordings.

We have achieved a number of refinements to the implantation technique for recording muscle electrical activity (EMG) by reducing size of the electrodes and connecting interfaces. This has helped to avoid potential problems with long term EMG implants.

Our research team places special emphasis on pre- and post-operative care of the highest possible standard, in consultation with the Named Veterinary Surgeon (NVS), who advises

the most appropriate pre- and post-operative analgesic and antibiotic regimes. We are also keen to adopt additional NC3Rs- approved refinements, where appropriate, to improve the outcome of the experiment and to improve animal welfare.

A retrospective assessment of refinement will be due by 28 July 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

8. Defining the molecular mechanisms underlying hypoxic ischaemic brain injury

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, pregnant, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of my group's work is the efficient discovery of mechanisms underlying brain injury which occurs in babies suffering a lack of oxygen to the brain during birth, and translating these discoveries into novel neuroprotective therapies. Such an ambitious aim can only be achieved through a considered approach which integrates *in vitro* and *in vivo* strategies to enable rapid translation to clinic.

A retrospective assessment of these aims will be due by 04 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?

- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Asphyxia (restricted blood flow/oxygen to the brain) during birth occurs in 2-3 term babies per 1000 in the UK, leading to the development of a condition known as hypoxic ischaemic encephalopathy (HIE) and permanent, life-long brain and motor disorders such as cerebral palsy. Following asphyxia, there is a delay of a few hours before the majority of brain cell death occurs providing clinicians with a valuable treatment window. The only available treatment, therapeutic hypothermia, is not always successful and new treatments are urgently required. However, therapeutic hypothermia does prove that intervening with treatment after the injury has occurred and within the treatment window can be effective. This project is designed with the overall aim of identifying novel therapies to combat the devastating effects of this brain injury. Over the next 5 years, we will strive to improve our understanding of the cellular mechanisms underlying the evolution of the injury, enabling us to generate significant and realistic avenues for therapy development ready for preclinical testing. Primarily and most importantly, the success of this project will have far-reaching, long-lasting improvements on the lives of significant numbers of babies and their families but in addition, the basic science outlined in the project will be of substantial interest to all researchers in the field of brain development.

What outputs do you think you will see at the end of this project?

The outputs from this project are extensive and focus on identifying mechanisms leading to mitochondrial damage and using these data to provide new avenues for the targeting of treatments. Mitochondria are structures contained within all brain cells which provide the energy required for cell survival and which are susceptible to damage following birth asphyxia. Using a variety of methods, we will generate large datasets of candidate molecules and novel pathways leading to mitochondrial dysfunction and will make these available to the wider scientific community using the appropriate platforms.

The spectrum of injury experienced by babies following birth asphyxia ranges from mild, which frequently is left untreated, through moderate to severe, for which there is currently no treatment; the prognosis for these children is bleak. We will be using a model which can encapsulate the range of outcomes will allow us to develop an understanding of which pathways contribute to the more severe outcomes and target therapies accordingly.
We will have generated a significant body of basic mechanism data contributing to the understanding of mitochondrial biology in the immature brain. We will also have provided a solid foundation for the development of therapies aimed at preventing mitochondrial-mediated cell death triggered in response to this injury.

These outputs will be disseminated through publications and used to underpin further grant applications. If applicable, we will also consult with technology transfer colleagues to pursue any therapies in collaboration with pharmaceutical companies.

Who or what will benefit from these outputs, and how?

Birth asphyxia is the second most common causes of death and disability in children under the age of 5 years in 2010, resulting in the loss of 50 million Disease Adjusted Life Years (DALYs). As there is currently only one therapy for this injury, which improves outcome for only 1 of each 7 infants treated, there is a critical unmet need. Our project is designed with the overall aim of identifying novel therapeutics to combat the devastating effects of term brain injury and there are wide-ranging short and long term benefits which may arise on successful completion of our project.

Short term, the basic cellular science outlined in the project will be of substantial interest to all researchers in the field of brain development and mitochondrial biology. We will also identify potential therapies through repurposing existing drugs or developing novel mitochondria-based interventions. Depending on the nature of these compounds, we will engage pharmaceutical companies in order to facilitate required testing prior to clinical trial.

Long term, and most importantly, the success of our project will offer far-reaching, long-lasting improvements in the lives of significant numbers of babies and their families who suffer the devastating consequences of birth asphyxia.

How will you look to maximise the outputs of this work?

We will maximise the outputs of this work in a number of ways.

We will provide our data to the scientific community through presentations at conferences dedicated to perinatal brain injury (e.g. Hershey conference on Developmental Brain Injury, June 2020). These are necessary in order to generate new collaborations depending on the focussed areas into which our experiments lead. We will place our large datasets in appropriate repositories for use by the wider scientific community and will publish robust data (including any *in vivo* negative data) in well-respected, open access, peer reviewed journals in the field.

Species and numbers of animals expected to be used

- Mice: 3000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We are using mice for this project. Mice are easy to handle and a wealth of information is already known about their genetics and physiology which assists in the interpretation of data and the planning of future experiments. Brains of mice continue to develop after they are born so therefore we will be predominantly using mice at the life stage of post-natal day 9 as this is the point at which their brain development is closest to that of a human newborn at full term. The mouse model of hypoxicischaemic brain injury outlined in this protocol is highly relevant to the human condition as it was used in the development of therapeutic hypothermia, currently the only therapy available to babies who have suffered from asphyxia during birth.

Typically, what will be done to an animal used in your project?

Mice will be bred in social housing conditions and may be ear notched for identification purposes.

Typically a post-natal day 9 mouse pup will be anaesthetised and undergo surgery for a maximum of 5 min and then allowed to recover in a warmed recovery box until the rest of its littermates have been through surgery. The entire litter is returned to the mother for an hour, whereupon the pup is then placed in a warm low oxygen chamber for 30 - 50 min. During this time, the pup may experience seizures similar to those experienced by the human newborn following birth asphyxia. However these do not last once the pup is removed from the chamber. The pup will then be left alone, given an injection of a neuroprotective drug and/or cooled down to 33C for 5 hours. This hypothermia does not cause the pup any pain or suffering. Pups remain with the mother until weaning when they are subsequently maintained for non-invasive behavioural experiments or until the experimental time point of interest. Each mouse will experience this protocol only once.

What are the expected impacts and/or adverse effects for the animals during your project?

All pups undergoing surgery will be regularly monitored.

Ischaemia requires anaesthesia and carotid artery ligation, which can occasionally result in the death of the mouse pup (<5%). During surgery, exposure to hypoxia may cause seizure-like behaviour, which rarely lasts beyond the duration of the hypoxia (30-50 min). Within the period of the previous licence, no animal experienced seizure activity following cessation of hypoxia. However, seizure activity in pups is associated with an increased risk of death and based on our recent experience, this would not exceed 15%.

We will mitigate against these harms by continuing to improve our surgery procedure to reduce anaesthesia time. We will also use the minimal time required to induce hypoxic injury (currently reduced 30 min) to limit exposure to the low oxygen environment. Initially, following surgery, these mice will generally have a low level of weight loss but the weight gain trajectory is entirely normalised within a week. After this time, it is difficult to distinguish the experimental animals from the control, untreated animals.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All genetically altered mice that are being bred and maintained for the projects will experience mild severity only. Mice in protocol 2 will experience severe severity as they undergo general anaesthesia, carotid artery ligation and exposure to hypoxia. The overall combination of these procedures means there is a risk of death up to approximately 20% during and immediately following completion of the protocol (within 1h). However, by 24h post surgery, subsequent recovery is usually in the mild severity category and the behaviour of mice is largely indistinguishable from their control litter mates. There are subtle behavioural differences, for example, forepaw preference can usually be observed in mice following the surgical procedure.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 04 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our project aims to understand the mechanisms involved in perinatal hypoxic-ischemic and infectious/inflammatory brain injury and to test neuroprotective strategies. As such, we need models that allow us to mimic human perinatal brain injury. Animals have to be used, as to validate a mode of action, experiments are required that cannot be conducted in humans for ethical and scientific reasons. In addition, interaction of the biological systems in whole organisms, with intact physiological barriers and excretion mechanisms, is key to inferring the potential of candidate therapies.

Which non-animal alternatives did you consider for use in this project?

We have considered the feasibility of achieving our purpose by not involving animals at all, for example by using cell lines or in vitro recombinant methods, but no such alternatives are able to reproduce the brain injury we aim to investigate in this proposal.

However, where possible (for example, in altering gene expression *in vitro* or for testing the specificity of pharmacological activators/inhibitors), we will replace whole animals studies with primary cell preparations or experiments in appropriate cell lines (e.g. neuronal SH-SY5Y, microglial BV2, oligodendrocyte CG4 lines).

Why were they not suitable?

Our project ultimately aims to identify neonatal neuroprotective strategies formulated from evidence using *in vitro* cell systems. However, *in vitro* systems alone cannot mimic the unique and complex environment that exists within the neonatal brain. The brain is comprised of many cell types and *in vitro* systems cannot model the physiological interactions and communication between diverse cell populations. In addition, we are aiming to discover therapies beneficial to the neonatal brain, the environment and developmental trajectories of which are still being determined. Therefore to generate clinically relevant data, *in vivo* neonatal models must be used.

A retrospective assessment of replacement will be due by 04 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have used power calculations based on our previous licence and through refinements to the protocol.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We regularly use PREPARE guidelines and NC3R resources such as the Experimental Design Assistant tool to make sure we are adequately powering our experiments while minimising animal numbers. We also use the services of the RVC chartered statistician and subsequently follow ARRIVE guidelines for publication of *in vivo* data.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The RVC has an efficient BSU with highly trained staff which will streamline the breeding and maintenance of genetically altered and wild type mice (protocol 1). In addition, the methodology of protocol 2 has been refined over a number of years and is in routine use in the lab. For neuroprotection studies we will plan pilot studies according to the guidelines on the NC3Rs website.

A retrospective assessment of reduction will be due by 04 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will predominantly be using mice at an early life stage. The brains of mice develop late in relation to birth and at postnatal day (P)9-P12, mouse brain development corresponds to the term human brain. Importantly, they share several important features with the human brain with regard to brain complexity and injury response in white and grey matter and thus can be considered a valid model in which to deliver the objectives of the project.

Why can't you use animals that are less sentient?

There are other animal models we could use, some in which the brain structure is more similar to that of a term human (primates, piglets). However, we have decided to replace the use of such animals with mice, without detriment to the science. Mice are considered less sentient at an early age, easier to handle, breed easily and have a short generation interval. In addition, much is known about their genetics and physiology.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Refinements we propose to test will include increased monitoring of vocalisation following surgery as a measure of distress in very young animals, in which standard characteristics of suffering or pain may not be so obvious. Equally, for young animals we will minimise rejection by the mother by rubbing the hands of the experimenter in bedding prior to handling the pups, to reduce transfer of unfamiliar smells. To this same end, routine monitoring will largely be through the side of the cage without disturbing the animals. Opening cages and handling animals will be limited to once daily as part of the standard observation and behavioural testing procedures within the project, unless a symptom of pain or distress is observed under which circumstance it may be appropriate to increase the frequency of monitoring.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We have followed the published guidance of a collaborator and leader in the field, Prof Henrik Hagberg, who refined the hypoxia-ischaemia procedure for mice including adaptations for use in P4/5 mice (Hagberg *et al*, 2002, Albertsson *et al*, 2014). We rely on Prof Donna Ferreira's recent study when adapting the surgery for strain-specific differences (Sheldon *et al*, 2019). We and others have also noted sex-specific differences which have further influenced our subsequent experiments (Mirza *et al*, 2015; Kichev *et al*, 2018).

References

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Mirza MA, Ritzel R, Xu Y, McCullough LD and Liu F. (2015) Sexually dimorphic outcomes and inflammatory responses in hypoxic-ischemic encephalopathy. J Neuroinflammation. **12** 32.
Sheldon RA, Windsor C and Ferriero DM. (2019) Strain-Related Differences in Mouse Neonatal Hypoxia-Ischemia. Dev Neurosci. **40** 490-496

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will stay informed through the NC3Rs website as well as taking advantage of the advice provided by the NC3Rs Programme Manager who will be based at the RVC one day per week.

A retrospective assessment of refinement will be due by 04 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

9. Detection of botulinal toxin

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Detection of Clostridium botulinum spores toxin

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Food producers are obliged to ensure the microbiological safety of all food supplied for human consumption (Food Safety Act, 2001). Food contamination by *Clostridium botulinum* can have severe and possibly fatal effects on consumers (man and animals) and is an important consideration in production and processing of foods and feed. As *C. botulinum* is ubiquitous in the environment, detection of low-level contamination, particularly in foods destined for vulnerable groups of the population (e.g. infants) is an occasional but important need. In the UK hazelnut yoghurt outbreak in 1989, 27 people were affected and 14 people were affected in 2007 when commercially produced chilli products became contaminated. The oral lethal dose for an adult human is very low (5 ng) which means a very sensitive test is required when an outbreak is suspected. The MLA is the most sensitive and appropriate assay available with current technology.

The objectives of this project are to

Examination of food samples suspected of containing botulinal toxin/spores (following suspect processing failures or suspect botulism cases).

Suspect botulinal cultures isolated either in-house or by other food testing establishments or clinical isolates.

REDACTED

Examination of animal feeds (consumed by food producing animals) for the presence of *C. botulinum* spores/toxin.

A retrospective assessment of these aims will be due by 27 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Examining products suspected of being contaminated with C. botulinum and/or its toxin or examining clinical isolates suspected in cases of botulism, will help identify the contamination source and prevent further infection/intoxication by others. Examination of food samples will also help to reduce food wastage and/or enable appropriate disposal of contaminated products, thus reducing impact on the environment.

Advancing analytical technology is closing the gap between in vitro and in vivo methods. Should sensitivities indicate a possible move to in vitro models during the licence period, some mouse lethality assays may be required to finalise method validation to ultimately replace the animal- based bioassay.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Mice, young adult, up to 1000. Only 8 samples have been tested since 2010. Large numbers are only used in the event of an outbreak situation. Four mice are used per sample and four mice for each control.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

After intra-peritoneal injection of the sample extract mice are often subdued due to the injection for approximately 15-30 minutes, after which they recover fully.

Botulism toxin causes progressive paralysis and death through respiratory failure if humane endpoints are not applied. Mice are observed frequently (at least hourly for the first 24 hours), from experience, if symptoms do occur then they will normally appear in

first 24 hours. If preliminary signs of botulism are observed (slight pinching of waist, rapid breathing) checks are increased until confirmed signs of botulism are observed (increased pinching of waist, cheyne stokes respiration, laboured breathing) at which point animals are killed humanely immediately. At the end of the test (3 days) all remaining mice are killed humanely.

Some mice (controls and spikes) may experience a severity level of moderate through exhibiting typical signs of botulism, however the majority will only experience a mild severity level as relatively few samples are found to be positive. The mice are checked regularly to ensure minimal suffering from botulism and are humanely killed as soon as typical symptoms are determined.

A retrospective assessment of these predicted harms will be due by 27 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

Alternative in vitro detection assays i.e. immunoassays, endopeptidase assays, mass spectrometry and cell based assays have significantly progressed over the last 10 years although no single method has emerged that can detect all botulinum neurotoxins in food at levels similar to those achieved by the MLA (mouse lethal assay). Most alternative methods do not detect all toxin types, and many do not detect active toxin. Low levels of toxin may not be detected by in vitro screening techniques but will be positive with the MLA. This is particularly important when you consider the oral lethal dose for an adult human is as low as 5 ng (5000 pg) of toxin for some strains of *C. botulinum*. The mortality rate for botulism is still 5-10% despite modern therapy which is still high for a food borne illness. Rapid treatment with antitoxin is an important factor in reducing the fatalities and severity of illness, so rapid accurate sensitive tests are of paramount importance. Where possible non-animal alternatives (NAA) will be explored and if found unsuitable then the bioassay will be considered. At present NAA’s would be appropriate where high levels of toxin are expected. This would allow effective sample screening with an in vitro method, but confirmation may still require the MLA. Negative samples would require further testing with the MLA to ensure toxin levels were not too low to be detected by the alternative method. The decision to perform the MLA would be made on a risk assessed basis and with consideration of the AWERB.

A retrospective assessment of replacement will be due by 27 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

The current method in use for the detection of *C. botulinum* or its toxins is the mouse bioassay. The number of animals used is the minimum number of animals required by this published method (4 per assay and four for each control).

Toxin typing is only carried out if required following a positive result. It is never undertaken without the confirmed presence of toxin present.

We do not quantitate toxin levels thus further limiting the number of mice used.

Ethical approval is sought prior to any testing to ensure that no unnecessary testing is carried out.

A retrospective assessment of reduction will be due by 27 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The mouse bioassay is a regulated, recognised published method for the detection of botulinal toxin and details the choice of species to be used. This method uses the minimum number of animals to give the results required to determine the presence of botulinal toxin. Animals are observed frequently (at least hourly for the first 24 hours and then 4 times/day for remaining test) for typical signs of botulinum. If preliminary signs of botulism are observed the frequency of checks is increased (to every 20-30 mins) until confirmation of botulism. Once confirmed signs are observed, animals are killed immediately by a humane method. Experience has shown that if symptoms do not occur during the first 24 hours, they are unlikely to occur.

A retrospective assessment of refinement will be due by 27 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

10. Development of Bacteriotherapies for Cancer

Project duration

1 years 6 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Cancer, Immunology, Therapy

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

It is now understood that our digestive system has an entire ecosystem of microbes that are essential for normal health and when this is altered, in the case of taking antibiotics, it can lead to the development of other conditions. We have identified that the mix of microbes varies greatly from person to person and that the specific make-up can influence the response to various treatments. A very recent finding has been that this can affect the development of cancer and also the response of cancer to many treatments we currently use. They might also explain the difference in the side-effects that patients experience.

We have begun to identify particular types of microbes, in particular bacteria, that correlate with the response a cancer patient has experienced to treatments. We aim to use this knowledge to develop cocktails of bacteria that could eventually be given to patients to either help treat cancer directly or to boost the activity of other cancer therapies.

Retrospective assessment

Published: 27 January 2022

Is there a plan for this work to continue under another licence?

Yes

Did the project achieve its aims and if not, why not?

The programme of work was completed although due to COVID-19 and restrictions in the facility and laboratory for social distancing the programme was modified to be achievable. The work will resume under a new PPL (PP8479036) at a new establishment.

The initial objectives were as follows:

1. To establish if candidate bacteriotherapies can prevent or reduce tumour growth in mice either alone or synergistically with existing immune-oncology therapies such as checkpoint inhibitors.

2. To perform preliminary mechanism of action studies of candidate bacteriotherapies in the absence of a tumour

It is anticipated that over the lifetime of this project licence it will

- Support development decisions regarding potential novel bacteriotherapies
- Lead to the identification of 1 or 2 lead bacterial consortia which may have a positive impact on response to immune checkpoint inhibitors to progress into further studies and ultimately clinical trials

Due to COVID restrictions the PPL was amended to remove aim 2 to enable focus on obtaining robust data for the therapeutic effect on the bacterial therapies identified and to obtain sufficient information for clinical trials to be started in 2022.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The significant shorter-term output of programme of work will be to generate data to demonstrate bacterial cocktails can be used to progress to pre-clinical development in preparation for clinical studies in humans. In the longer term, we expect our lead novel bacteriotherapies to treat cancer in humans to reduce morbidity and mortality. The results of the research will be published in scientific journals and presented at scientific conferences. New mouse models may be patented and shared with other researchers.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Over the 18 month period of the project, we anticipate to use 920 mice.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

Up to 120 mice will be used in breeding to generate the experimental mice we require. Up to 700 mice will be used in our cancer studies and will experience moderate severity as we have to handle them more in order to carefully monitor the tumour growth. They will be injected with cancer cells to their side to generate a small lump and are measured regularly to track lump size and mice are humanely killed before the product of maximum length and maximum width of lump reaches 1.2cm2. The length and width of the lump size will be measured using callipers. These mice will also be colonised with particular bacterial species either using samples obtained from human cancer patients or what we have grown in the lab. Sometimes in order to help these bacteria grow we first need to give the mice antibiotics either in drinking water or via intra-peritoneal injection to eliminate the bacteria that already live in their digestive tract. At various times they will be treated with our candidate bacterial cocktails given via oral gavage by putting a small tube into their throat for a very short period of time to deliver the bacteria into the stomach. We will use the least invasive way possible to administer the antibiotics some of which can be in the food and drinking water but occasionally due to the antibiotic we need to do this by injection. In general the bacteria needs to be administered via oral gavage as we are using anaerobic bacteria that can grow in the absence of oxygen and ensures the delivery to the correct location. A subset of these mice will be used in our therapy studies and will be administered via intra- peritoneal injection with anti-cancer drugs such as those treatments that are currently used in hospitals to treat cancer patients. All these procedures will cause minimal suffering to the mice.

All the mice will be check daily by qualified technicians. A small number of mice who are cured of their cancer and the small lump is no longer detectable will be injected again with the same cancer cells to their opposite side. This may allow us to show that the effect on the immune system is long lasting and represents good candidates for long-term therapeutic benefit to cancer patients. When the mice reach the endpoint of the study, defined by a humane endpoint such as tumour size or timepoint, they will be humanely killed prior to tissue collection. Some mice could have small blood samples collected while they are alive so that we can track the effect of a treatment or larger volumes collected after they are humanely killed. Mice typically do not show any altered behaviour when administered cancer cells, however on some occasions they may not have control of the cancer cell growth or the immune response can be so effective it leads to other side effects such as nausea also seen in cancer patients. We select the cancer cells to be administered to be the most suitable for our experiment which give rise to masses that are well tolerated. Very rarely the mice may scratch at their lumps causing the skin to be broken and if this is observed the mice are humanely killed. Some of the immune system treatments, as they are designed to provoke the immune system, can give rise to symptoms such as increased temperature and diarrhoea. This will be closely monitored (twice a day or more if necessary) and when it exceeds certain thresholds the affected mice will be humanely killed.

The final 100 mice will be used for studies without a tumour to understand how the bacterial cocktails interact with the immune system to cause the therapeutic effects that we observe. Half of these mice will experience mild severity as they will be colonised with the bacterial cocktails prior and subjected to a small number of injections of immune system altering agents. The remaining mice will experience moderate severity as they will be administered the bacterial cocktails and immune system altering agents on multiple occasions. Some mice could have small blood samples collected while they are alive so

that we can track the effect of a treatment or larger volumes collected after they are humanely killed. These mice could experience some side effects from the treatments which is quite similar to a bad cold, they are carefully monitored and if they exceed the criteria they are humanely killed. We will provide extra bedding and additional support to the mice to minimise these effects. The models and experimental protocols that we are using are well established, widely used and designed to cause the least pain, suffering and distress to the mice.

Retrospective assessment

Published: 27 January 2022

What harms were caused to the animals, how severe were those harms and how many animals were affected?

A total of 104 germ free mice were used in breeding with 95 of these experiencing sub-threshold severity, 7 mice had moderate severity and culled sick with suspected caecal torsion and 2 severe due to being found dead as a result of caecal torsion which was confirmed on necropsy. A total of 430 SPF mice were used in therapeutic studies where they were given a tumour on their flank by injection of mouse cancer cell lines and exposed to potential therapeutic bacteria or a control substance by gavage and also a clinically approved immune based treatment or control was injected. Of these 430 mice, 1 was found dead after treatment with bacteria and would have had a severe experience, the remaining 429 would have an overall moderate severity due to the cumulative effect of the procedures performed with tumours allowed to reach 1.2cm2 as the endpoint. A further 130 germ-free animals were used in our colonization therapeutic studies and were given a tumour on their flank by injection of mouse cancer cell lines and exposed to potential therapeutic bacteria or a control substance by gavage and also a clinically approved immune based treatment or control was injected. Of these mice, 1 was found dead after treatment with bacteria, the remaining 129 would have an overall moderate severity due to the cumulative effect of the procedures performed with tumours allowed to reach 1.2cm2 as the endpoint.

Replacement

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

The cells of the blood system and their interaction with tumours is very complex and requires the interaction with other cell types for which it is not possible to use a non-animal alternative. Also, it is not possible to add in the extra complexity of the microbes using in vitro methods as these contain agents to stop the microbes growing. We will use existing data sources rather than duplicating where these exist and also harvest additional tissues for use in alternative lab-based experiments where possible. Should new lab-based or computer-based models exist which generate comparable data these will be adopted.

Retrospective assessment

Published: 27 January 2022

What, if any, non-animal alternatives were used or explored after the project started, how effective were they and are there any lessons worth sharing with others?

Where possible we have utilised microbiological culturing techniques and human derived tissue cultures to identify the behaviour of bacterial species and replace the need for early mechanistic studies in animals. We estimate that this resulted in a reduction of 90 mice that did not need to be used as the cocktails had already been refined and did not require in vivo investigation.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We will use various statistical approaches to determine the minimum number of animals to use in an experiment and where possible combine experiments. We will also harvest additional tissues from mice at the end of experiments to use in laboratory-based assays to reduce numbers needed. Proper design of the experiments and controlling for sources of variation such as the age and sex of the animals will also help increase the robustness of the experiment and result in an overall reduction in animals needed. When publishing our data we will follow the ARRIVE guidelines to ensure comprehensive reporting and will release all data including where we do not find any alterations as this can be as informative to prevent assays being repeated in other laboratories. The results of the research will be published in scientific journals and presented at scientific conferences.

Retrospective assessment

Published: 27 January 2022

How did you minimise the number of animals used on your project and is there anything others can learn from your experience?

Through the improved transplantation and the ability to use SPF mice we were able to reduce the number of germ free mice that were needed to be used to complete the required studies. This transplantation method was based on Routy et al Science 2018 and will be published in due course.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We use mice for select experiments as they represent an ideal model to study host-microbe and immune cell interactions and serve as an invaluable pre-clinical model for anti-cancer therapy development. The use of mice in cancer studies is well established and the models we plan to use are considered 'gold standard' and are used in the development of therapies that are now used in the clinic and resulting in good responses in some cancer patients. In all our experimental work we will minimise the number and severity of procedures applied to the mice, this could be via the selection of the substances that we administer causing the least number of side effects, administering substances together if possible. We will use cancer cells that are pathogen free and that will not make the mice sick and will investigate alternative methods to monitor the growth of the cancer cells in the mice to minimise stress from handling. When we are performing experiments requiring mice to be anaesthetised for a period of time we will use agents that

allow for a rapid recovery and that will not affect the mice. As we can monitor the survival of the bacteria in the mice via collection their poo we can minimise the number of administrations of bacteria via oral gavage. Also, we will investigate the possibility to colonise mice by co-housing as the microbiome can be transferred in this way or via bedding from other mice. The substances that we administer to treat them will be of the purest grade and screen for possible contaminants that could mice sick.

The use of a sophisticated mouse tracking system allows accurate tracking of all health concerns associated with the mice to be used in this study and to enable rapid investigation where they occur at a higher than expected incidence. All people who work with the mice in this study are thoroughly trained and continuously assessed for their ability to perform these procedures, with procedures refined following advice from the vet/NACWO or other international guidance. In addition, a comprehensive staff training and competency assessment process is implemented for all animal users within the establishment with continuous evaluation of procedures to ensure they are performed optimally or adjusted to benefit animal welfare. Mice are group housed in an enriched environment.

Retrospective assessment

Published: 27 January 2022

With the knowledge you have now, could the choice of animals or models used have been improved at all? How did you minimise harm to animals during the project?

Improvements to germ free animal breeding and maintenance including reducing the number of litters and maximum age of the breeders to reduce incidence of mice affected by caecal torsion. Females were allowed to have three litters although due to the poor breeding performance this was generally 2 litters. Males were removed after the second litter with the maximum mating duration of 14 weeks and maximum age of mice 24 weeks. The age of mice entering procedural work or held as stock was capped at 12 weeks again to reduce the incidence of germ free mice being affected by caecal torsion. All germ free stock animals were checked twice per day in order to better spot clinical signs and allow earlier intervention.

An improved protocol was introduced to enable SPF mice to be reproducibly colonized with human microbiome or bacterial cocktails after limited pre-treatment with antibiotics, this was based on Routy et al Science 2018 with details to be published in due course.

11. Development of novel therapies for the treatment of stroke

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The central aim of our research is to develop new therapies for stroke and the complications of stroke such as chronic pain.

A retrospective assessment of these aims will be due by 18 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Stroke is brain injury caused, in most cases, by the blockage of a blood vessel that supplies blood to the brain. There are approximately 150,000 strokes per year in the UK and it is the largest cause of adult disability. There is currently only one approved acute pharmacological therapy, tissue plasminogen activator (TPA) which must be administered to stroke victims within 4.5 hr of symptoms onset. However, only 3-10% of patients will receive this medication primarily because most do not arrive in hospital early enough to be eligible; or because they are taking medications that make them ineligible for the clot busting therapy. Thus, there is a desperate need for new therapies.

What outputs do you think you will see at the end of this project?

The development of drugs which have multiple mechanisms of action. The information from our work could potentially be used to design studies in humans. We would publish our findings in peer- reviewed journals and present at national and international conferences.

The development of a vaccine for stroke. This would allow the treatment of large numbers of patients who are at risk for stroke. The information from our work could potentially be used to design studies in humans or design monoclonal antibodies or drugs. We would publish our findings in peer- reviewed journals and present at national and international conferences.

To better understand the body's own protection mechanisms against stroke and to understand how these messages may be transmitted to subsequent offspring. This knowledge could be used to develop new therapies. If we are able to identify new pathways that are involved in conditioning, the information from our work could potentially be used to design new neuroprotective drugs. We would publish our findings in peer-reviewed journals and present at national and international conferences.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

If we are successful in developing a new therapy for stroke, this could have huge benefits for humankind. Stroke is the third biggest cause of mortality and the biggest cause of adult disability worldwide. A new therapy for stroke that could benefit large numbers of patients is desperately needed. We also have the potential to help individuals who develop complications of stroke such as chronic pain.

By the end of this body of work (5 years), we hope to have identified:

1-2 new drugs, that could be ready for human testing

Tested a vaccine for safety and efficacy preclinically, ready for advanced toxicology studies.

Identified mechanisms that underlie conditioning. This information could be used to design new drugs that target those pathways.

How will you maximise the outputs of your work?

Publication of results in peer-reviewed scientific journals will be a major element in the dissemination strategy of this body of work, therefore making the research available for exploitation by stroke and pain researcher worldwide. Additionally findings that will result from this proposed body of work will be disseminated by presentations at national and international conferences.

Furthermore, in order to widen the scope for knowledge transfer, visits and collaborations with other laboratories in the UK, Europe and/or USA will be organized during the duration of the project. I have very strong relationships and ongoing collaborations with several laboratories worldwide and my laboratory team members will be encouraged to visit these laboratories.

Species and numbers of animals expected to be used

- Mice: 3200

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

The majority of the animals that will be used in this project will undergo induction of ischemic stroke. Some animals will undergo transient or permanent occlusion of the middle cerebral artery (MCA) by inserting a nylon microfilament into the MCA, through the carotid. Some other animals will undergo permanent MCA occlusion by direct occlusion of the MCA by eletrocoagulation. Some other animals will undergo MCA occlusion by injection of a clot into the MCA through the carotid.

All animals will be tested for functional outcome, including neurological evaluation, motosensory evaluation, development of pain and cognitive impairment.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Following transient and permanent MCAO, animals will show transient weight loss and neurological impairment as a result of the surgery and the experimental stroke. Activity can be subdued for a few hours after recovery from anaesthesia. The weight loss recovers around 48 hours. The functional impairment starts to improve significantly after 48 hrs and is very mild after 72 hrs and specialised functional tests such as the Garcia scale are used to detect the functional deficits because they are so mild.

About 30% of animals are expected to be sacrificed at 48 hours. The rest of the animals will survive to about 30 days post-surgery to look for long term functional improvement.

We have recently discovered that animals develop a mild hypersensitivity to pain in the affected limb which appears to persist to about 30 days. However, this pain and is mild and does not appear to affect activity, feeding or other behaviours.

The therapeutic agents that are being tested could potentially have toxic effects which may manifest in weight loss, reduced activity and feeding but we have not encountered such effects with the agents we have tested. If toxicity is encountered then these animals will be culled.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The stroke model we are using mimics the human disease. After induction of stroke, animals will develop neurological deficits. Most of the transient middle cerebral artery mice are expected to reach severe level early after surgery (48hrs) and recover very well after that and have little observable deficits subsequently. In the permanent stroke models the deficits are very mild and mice have excellent recovery - all these mice reach moderate severity. All animals will be closely monitored and any animals exceeding the expected severity level will be terminated.

For the PK studies, the mice are expected to reach mild to moderate severity only.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 18 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The overall objective of this project is to develop, new therapies for acute stroke. We have used *in vitro* cell culture models and other *in vitro* assays to identify candidate molecules in previous and ongoing studies. Those studies allow us to select the best candidates for testing in animals.

For approach 1, animal experiments are needed to optimise and determine the best dosing that will be required for efficacy and safety.

For approach 2, previous *in vitro* work has identified candidate target molecules for development. We now need to test efficacy and safety in animals.

For approach 3, we need to confirm our preliminary studies that appear to show transgenerational protection. Candidate mechanism will be explored using *ex vivo* and *in vitro* approaches wherever possible but it is inherently impossible to do transgenerational studies using cells.

What was your strategy for searching for non-animal alternatives?

The most frequently used in vitro model of cerebral ischemia is the combined oxygen and glucose deprivation (OGD). Retaining glucose in the hypoxic chamber is less suitable for modeling an ischemic event, which always is accompanied by breakdown of the nutrient supply. Additionally, Compared to *in vivo* models, there is a need for a longer episode of energy deficiency to induce neuronal death.

Another way to model ischemia in vitro is the organotypic brain slice. This approach has the advantage of a functional system with preservation of the neuronal morphology and the presence of glial cells and network connections in particular when using hippocampal slices. However, the lack of perfused vessels in brain slices clearly represents an artificial situation, despite the fact that allows separation of the ischemic effects on neuronal tissue from those due to actions on the cerebrovascular system.

In vitro models allow the investigation of specific basic biochemical and molecular mechanisms under conditions of energy deficiency similar to ischemia. The fundamental critical control points and molecular pathways of necrotic cell death, programmed cell death and autophagy are also amenable to direct study in vitro. However, the complex situation of ischemic stroke cannot be modeled in an *in vitro* system with single cells or pieces of brain tissue with the absence of intact blood vessels and blood flow as well as the lack of infiltration of leukocytes.

Why were they not suitable?

Although *in vitro* experimental research has been an important component of our overall development plan, it is not, however, sufficient on its own because stroke involves complex interplay of multiple mechanisms in various organs. For example, the peripheral immune system as well as other inflammatory processes outside the CNS can influence the integrity of the blood brain barrier which is an important barrier against stroke injury. This can only be tested in living animals. Furthermore, an important part of stroke efficacy and safety evaluation is the study of functional and behavioural outcomes which is not possible using *in vitro* experiments or computer simulations.

A retrospective assessment of replacement will be due by 18 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise

numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

A statistician has provided input in study design and statistics.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

The number of animals will be kept to minimum by ensuring that the experimental designs to be used are rigorous and that all personal licensees (and dedicated animal care staff) working on this project are appropriately trained and suitably competent. This will enable a high success rate to be achieved with minimum number of animals being used.

Initially, we will test efficacy at 24 hr and only if the efficacy is observed at 24 hours will the chronic experiments looking for efficacy and safety at 28 d will be carried out. This will reduce the number of animals that will be exposed to the experimental protocol for 28 d. Chronic testing is essential because it is possible that our candidate therapeutics may be efficacious at 24 hrs but this protection is not sustained over the longer term.

What other measures apart from good experimental design will you use to minimise numbers?

Wherever possible, we will use published literature (and if more appropriate conversations with colleagues in the field) to guide any experiments. For example, choice of a starting dose will be based on previous experience with that drug and analogues. We will also do pilot studies for new drugs.

Wherever, possible, we will use *in silico* modelling to assess toxicity and potential organ toxicity of a drug before starting experiments.

A retrospective assessment of reduction will be due by 18 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Choice of species

An experimental stroke model requires an animal with an intact cerebral circulatory system. In order to mimic clinical stroke lesions, we require an experimental species high on the evolutionary tree. Mice are a common and desirable choice of mammalian species and are widely used in scientific research. Their cerebral vasculature is relatively well documented and in many ways resembles the Circle of Willis in man. The models proposed here are standard ones used by many previous workers in this area of research including the applicant who has over 20 years of experience with this model.

The models

The MCAO model of ischemic stroke is the primary model used in experimental stroke research.

The methods proposed here involve the intraluminal filament method whereby a coated filament is introduced in to the common carotid artery and advanced along the internal carotid artery to occlude the middle cerebral artery (MCA). For transient ischaemia, the filament is withdrawn after about 90 mins. For permanent ischaemia, the filament is left in place. The stroke can also be used by drilling a small hole in the skull and closing the main blood vessel.

A more recent modification of the model requires injection of preformed blood clot introduced via the carotid artery to occlude the MCA in an attempt to more closely model human stroke.

Although these models were originally developed in the 1980 and 90s, detailed analysis of these model and subsequent refinement have revealed that strict experimental control can be applied to reduce the variability of infarct size seen in other models. Thus, these models produce a reliable and reproducible infarct volume. This programme of research will use the transient and permanent model of MCA occlusion. To reduce variability, we will ensure that there is occlusion of the MCA using laser Doppler. In addition, we will ensure that core body temperature is maintained during surgery which can drastically affect the extent of neurological injury. There is, however, some inherent variability in this model. This is largely due to variability in the cerebral vascular anatomy and therefore collateral circulation.

Recently, a new paper (IMPROVE guidelines) has been published providing a step-by-step guide to improve animal welfare in stroke models. We will work with our veterinary surgeon to determine how we may implement some of these refinements in our existing stroke models to reduce pain, suffering and distress.

Severe limit protocols

To determine whether our experimental treatments can reduce the ischemic damage, it is necessary to create a sufficient size of brain infarction which will cause some functional deficits. These functional deficits include moderate degrees of deficits such as paralysis of the contralateral limbs, sensory loss and neglect. In some cases, the brain damage may cause mortality, although this is on occasions unavoidable, we aim to minimise any mortality by humanely killing any experimental animals which appear unlikely to recover.

In particular, any animal which shows persistent barrel rolling, respiratory distress or loss of the righting reflex will be killed humanely. We anticipate that less than 10 per cent of

animals undergoing surgery will either die spontaneously or exceed the severity limits and have to be culled.

All animals are carefully and regularly monitored. Post-operative monitoring sheets are completed and kept with the animals ensuring their progress can be identified by any member of technical staff. Data obtained from animals undergoing surgery indicate that roughly 20% of animals will show weight loss of 20-30% at 48 hrs.

Weight loss is undoubtedly a significant factor in the recovery following surgery and we implement a number of measures to promote feeding and drinking following surgery. Animals will receive fluid supplements via subcutaneous injection immediately after surgery. Subsequently, they will receive mash/wet gel. It is also useful to place these in the cages for a few days prior for the animals undergoing surgery in order for them to become acclimated to this food source. In addition, following surgery, movement of animals is restricted and loss of body heat it is a potential problem – although local heat can be supplied via the use of heating lamps, all post-surgery animal cages will be housed in an environment chamber which is beneficial for providing a constant source of heat.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

In order to properly mimic stroke preclinical we need to use species that are higher in the evolutionary tree adn show a central nervous system similar to the human one. Additionally the cerebral vasculature of rodents is relatively well documented and in many ways resembles the Circle of Willis in man, critical for stroke.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

We will closely monitor the animals following stoke induction, in particular for the first 72hrs, to make sure that the animals are properly recovering from the surgery despite the impairments that will occur following ischemia onset. Additionally, we will closely work with the NVS and NACWO to improve the outcomes of our procedure and minimise animal distress.

We will constantly refine the use of experimental animal models and reduce the impact on the animal. We are working on refining our surgical methods that are used to induce MCAO which can affect the results and also reduce the impact/severity on animals.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We will follow the STAIR guidelines and monitor for any updates as well continuously check the literature for any novel improvements to the animals welfare, for example the IMPROVE guidelines may provide additional steps to improve our animal models.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We will continuously check the literature, check the NC3Rs portal, and have conversation with the NC3Rs Regional Programme Manager .

Explain the choice of species and the related life stages

Previous work has used in vitro approaches to select candidate drugs and therapies which underpin the three approaches that we have proposed in this body of work. Our previous work has allowed us to select candidates with the highest likelihood of success. However, going forward, in vitro work cannot replace animal experiments which are needed to confirm efficacy and safety.

The outcome measures that need to be tested include functional and behavioural testing which cannot be tested in cells. In addition, stroke injury involves a complex interplay of many different cells in the brain and many organs systems. This cannot be tested using in vitro approaches. Therefore the need of performing in vivo experiment for which adult mice is the most appropriate species.

A retrospective assessment of refinement will be due by 18 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

12. Development of novel treatment options for REDACTED infection

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

There is currently no effective treatment for REDACTED infections (which include rabies virus) once the patient or animal has developed clinical signs. This project is to develop proof of concept for utilisation of novel live attenuated rabies vaccines for post-infection treatment (PIT) of rabies and other
REDACTED.

Retrospective assessment

Published: 03 November 2022

Is there a plan for this work to continue under another licence?

No

Did the project achieve its aims and if not, why not?

The three objectives under this license were:

1. Assessment of Safety of candidate post infection treatments (PIT)

2. Assess protection of the PIT when administered before and after natural virus infection but before pre-clinical timepoints.

3. Assess protection of the PIT when administered after initial signs of clinical disease.

Of these, the first two objectives (assessment of safety of candidate PIT treatments, and the assessment of protection of the PIT when administered before and after natural virus infection but before pre-clinical signs) have been undertaken.

The first objective of this PPL was described as “Assessment of Safety of candidate PIT treatments”. This objective was satisfied by the first in vivo experiment performed. Here, the PIT assessed was investigated for safety when administered to an appropriate animal model over a range of concentrations by either the intravenous or intracranial route. This study revealed that the PIT used was safe when administered both intracranially and intravenously at a maximum dosage of 104 FFU per dose. This indicated that the replication of this novel PIT was attenuated further over the backbone vaccine strain that the construct was based upon. Furthermore, experiments performed at the end of this study supported the suitability of the intracranial route and 104 FFU dose by demonstrating that the animal model gave a strong neutralising antibody response after challenge. Together, these results satisfy the first objective in assessing the safety of the PIT and demonstrated that the PIT appears to have a better safety profile that the viral construct that the scFv and mutated glycoprotein have been added to.

The second objective focussed on whether the antibody was able to protect against mortality if given before the onset of RABV clinical signs. In short, an in vivo study was performed to assess whether RABV- ****antibody could protect against mortality if given simultaneously with lethal RABV challenge, or three/five days after. Survival analysis of this study revealed that while survival rates decreased as time between lethal challenge and RABV treatment increased, a significant difference was seen in survival rates between the untreated-lethal challenge and Day 3 treated – lethal challenge groups. Furthermore, analysis of the clinical scores assigned to animals throughout this experiment revealed that RABV- **** was effective in delaying the progression of disease after lethal challenge.

The third objective of this PPL was to assess protection of the PIT when administered after initial signs of clinical disease. In short, this objective was not achieved. This was due to a variety of reasons. Predominantly, the survival analysis results from the second in vivo study indicated that while RABV-**** was effective in delaying the progression of symptoms and significantly increasing survival when administered after lethal challenge, it would not be sufficient to prevent mortality if given after the onset of clinical signs, in vivo studies to satisfy objective three were not performed.

In summary, the programme of work has not been fully completed, and continuation under a further project license is not currently envisioned as the results from the studies undertaken for the second objective indicated that while PIT was effective in delaying the progression of symptoms and significantly increasing survival when administered after lethal challenge, it would not be sufficient to prevent mortality if given after the onset of clinical signs.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these

could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Every year there are at least 59,000 human deaths associated with infection by rabies virus, 40-50% in children under the age of 14 years. Whilst effective vaccines and post exposure treatments exist, provision of vaccine and other important rabies post exposure prophylaxis (PEP) tools, e.g. human rabies immunoglobulin (RIG), are not available where they are needed most, generally due to the expense involved in manufacture across the developing world.

What outputs do you think you will see at the end of this project?

The in vivo assessment of various approaches to post infection treatments (PITs) for rabies and other REDACTED.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

If successful and subsequently developed these treatments would contribute to a significant reduction in the 59,000 human deaths that occur annually across the world.

How will you maximise the outputs of your work?

As well as scientific publication, the research group is member of various European and International collaborations, REDACTED

Species and numbers of animals expected to be used

- Mice: 900

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Unless controls all will be injected with PIT treatments (300), PIT treatments that show no adverse effects the second type of experiment will involve infecting mice with rabies as well (300). If the PITs are successful apart from the controls there should be no clinical effects of this infection, there are safeguards of humane endpoints for controls and candidate PITs that are shown by the experiment not to offer protection.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

There will be impacts around the methods injection of the PIT and which include intracerebral for which anaesthesia and pain relief will be given.

The PITs under investigation are based on replication competent rabies vaccines expressing heterologous genes. Although vaccine strains of rabies are generally avirulent when administered via the peripheral routes, disease can occur following intracranial inoculation.

Animals will be monitored following inoculation to assess any adverse effects of the PIT or procedure. If any adverse effects are seen that are deemed to be a result of the inoculation procedure then animals will be assessed and the outcome determined by advice from the NVS.

When rabies infection is given the impact will vary with at what stage of the experiment it is given. Initially rabies infection will be given after treatment with PITs, this will allow earlier humane endpoints to be used when initial signs of disease are seen. Candidate PITs that pass this stage will be used on mice showing early stages of clinical disease and because of this the mice will be constantly monitored a later humane endpoint maybe necessary.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

75% may be moderate or below, 25% will up to severe.

What will happen to the animals at the end of the study?

- Killed

Retrospective assessment

Published: 03 November 2022

What harms were caused to the animals, how severe were those harms and how many animals were affected?

Inoculation routes: Intercranial (IC) Intramuscular (IM) Subcutaneous (SC)

Blood sampling: From tail veins (<100 µl), and Cardiac Puncture (CP)

Use of anaesthesia: For IC, IM inoculation and CP

Use of analgesia: Subcutaneous (SC)

Capture: Using scoop technique

Volumes of substances: IC (30µl), IM (50µl), SC (150µl)

Inocula details: Live virus (IC and IM), PIT (IC), and vetergesic (SC).

Anaesthesia: inhalation of isoflurane

2020 – 118 mild & 75 moderate

2021- 60 mild & 216 moderate

Actual harms:

- Development of clinical disease by infected animals- regulated by clinical scoring and termination at predefined humane endpoints.
- Transient harm from inoculation- mitigated by use of analgesia and anaesthesia.
- Animals are monitored following inoculation to assess any adverse effects of the inocula or procedure. Adverse effects where seen were assessed with input from the NVS.

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The complexity of REDACTED infection spread and its impact on the central nervous system, the influence of animals immune system and impact of the blood brain barrier on efficacy of treatment may mean that animals have to be used.

What was your strategy for searching for non-animal alternatives?

The non-animal alternatives considered are given below and have been incorporated in the build up to the animal work.

The demonstration that both plant-produced and cell-culture produced ScFv are able to effectively neutralise rabies in vitro;

ScFv produced by novel rabies post infection treatment (PIT) preparations will also be shown to neutralise rabies virus;

Furthermore, wherever possible these novel rabies PIT preparations will be analysed in a novel blood- brain barrier model to confirm that the live-attenuated virus is able to cross the BBB to produce neutralising ScFv behind the BBB.

If ScFvs fail at any point they will not go onto the animal phase

Why were they not suitable?

They are useful to dissect individual aspects of the work but ultimately candidate ScFvs that have passed the in vitro assessment need to be tried in the more complex in-vivo model.

Retrospective assessment

Published: 03 November 2022

What, if any, non-animal alternatives were used or explored after the project started, how effective were they and are there any lessons worth sharing with others?

No non-animal alternatives exist to deliver these studies and so no replacement is possible. Full literature searches have been continually carried out during the project life alongside consultation with experts in both rabies and rabies vaccine design. The data that has been derived from the proposed study is not available elsewhere.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers are estimation based on an estimation of the number of candidate PITs thought to be generated and the experience of working with rabies vaccines previously.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

When the in-vivo work starts these will be refined using pilot studies, followed by confirmatory experiments designed to test specific hypotheses. Where appropriate, full power analyses (e.g. using nQuery Advisor) will be used to calculate sample size, taking into account the likely effect of size.
REDACTED

What other measures apart from good experimental design will you use to minimise numbers?

Experiments are staged (below), with candidate PITs having to pass each stage before moving onto the next stage. The in-vivo stages are below

Assessment of candidate PITs by different routes (without virus challenge) to ensure safety

Successful PITs administered before and after natural virus infection (pre-clinical signs).Then if they provide protection in this scenario.

The PIT administered after initial signs of clinical disease

Retrospective assessment

Published: 03 November 2022

How did you minimise the number of animals used on your project and is there anything others can learn from your experience?

A full statistical assessment of the planned study has been performed and advice has been taken to optimize the number and grouping of animals

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Mice are the biological system of choice for these studies. They are sufficiently immunologically similar to humans to allow confident extrapolation of findings, yet they are more amenable to laboratory experimentation at the organisation than dogs, cats or non-human primates which would otherwise be the species of choice for these studies. Further, physical size, social structure and husbandry requirements are conducive to humane care in containment laboratory settings.

It is becoming increasingly apparent that although rabies virus is considered to be 100% fatal following the development of clinical disease, exposure to virus will not always result in disease. Indeed, infection with lyssaviruses via different routes can result in different clinical outcomes. From established mouse studies, inoculation of neat preparation of virus via the IC route generally causes 100% development of clinical disease although rare exceptions occur where IC inoculated animals survive infection. As the IC route is invasive, applica. The mechanism behind survival following IC inoculation is unclear.

Importantly, even live attenuated vaccine strains, when administered by the IC route, can cause clinical disease whilst peripheral inoculation renders these viruses avirulent. This will need to be assessed to ensure safety following peripheral inoculation. Furthermore, even with clinical disease confirmed in IC inoculated animals, inoculation of the same virus via a peripheral route will often lead to a reduction in the number of animals that succumb to infection. For this reason, when trying to mimic natural routes of infection larger group sizes are required to ensure that enough animals develop clinical disease and can be studied closely. Those that do not succumb are assumed to have had an abortive infection whereby: 1) virus has replicated within the infected animal and is cleared by the host immune response and serological evidence of exposure is seen or; 2) for undefined reasons neither serological nor clinical responses occur. Different strains may be used for experimental studies to minimize variation within and between experiments.

REDACTED. Using this we will be able to keep the PIT treatment pre and post infection protocol to moderate severity as mice will be killed humanely at early signs of clinical disease. However on exceptionally rare occasions animals have been found dead on morning checks.

For successful PIT candidates it will be necessary to treat mice showing clinical signs of lyssavirus infection so this protocol we consider will be severe. Treated mice will be

monitored throughout the day at hourly intervals until as late as is possible in the evening and euthanased as soon as clinical score 2 is reached.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

To assess whether a PIT based on replication competent rabies vaccines expressing heterologous genes so a competent immune system is required, this involves using fully developed animals with associated sentience.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

As well as pre-start meetings involving the NVS, NACWO and animal care staff to ensure current knowledge is brought to bear, all projects are followed up by a wash up meeting. All aspects are discussed, was the project a success, what went well and if there was anything that could be done better. If there are any suggestions for refining the procedure they will be considered and if appropriate, incorporated into the protocol.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Home Office The Harm–Benefit Analysis Process
Home Office Guidance to ASPA
Home Office Code of practice
RSPCA Guidance on Welfare of mice

How will you ensure you continue to use the most refined methods during the lifetime of this project?

I have regular contact with the NIO, NACWO and NVS through various forums and use of the library function which can scan for relevant publications. In developing this work I have been in contact with other researchers outside of the organisation who also specialise in rabies disease work.

Explain the choice of species and the related life stages

Mice are the biological system of choice for these studies. They are sufficiently immunologically similar to humans to allow confident extrapolation of findings, yet they are more amenable to laboratory experimentation at the organisation than dogs, cats or non-human primates which would otherwise be the species of choice for these studies. Further, physical size, social structure and husbandry requirements are conducive to humane care in containment laboratory settings.

The established model uses mice at a few weeks of age, they are fully immunologically developed and do not have any issues of old age.

Retrospective assessment

Published: 03 November 2022

With the knowledge you have now, could the choice of animals or models used have been improved at all? How did you minimise harm to animals during the project?

Following numerous experimental procedures with lyssaviruses over many years, mice have been established as a good indicator species for lyssavirus infection. Several refinements have been made to protocols in this study. These are outlined below:

1) The use of translucent lids to transfer mice for inoculation has reduced the jumping activity of the mice which has reduced stress for both the mice and staff performing inoculations.

2) The use of analgesia before IC inoculation.

3) The purchase of IVCs has enabled mice to be assessed with minimal disturbance.

4) The use of ear tags to identify the mice in place of subcutaneous chips, and later, the use animal marker pens have reduced the stress to the animals and made it easier for scientists to identify the animals.

5) To avoid observational biases, studies have been blinded to remove biases when scoring clinical disease.

6) Following an unscheduled death of a mouse on another study, the refinement was taken to avoid certain enrichment in the period when mice are recovering from gaseous anaesthesia.

13. Redacted

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Horses	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

REDACTED

A retrospective assessment of these aims will be due by 14 July 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Veterinary surgeons and trainers will be able to make informed decisions about what therapeutic medication to use and when within regulatory rules.

REDACTED

Alternative *in vitro* methods will be developed wherever possible

What outputs do you think you will see at the end of this project?

REDACTED

Publications - frequent peer reviewed journals , information sections on relevant websites- governing bodies and equine veterinary and welfare organisations

REDACTED

Interaction with general public - we are a signatory to the concordat on openness on Animal research

Education of industry stakeholders through presentations, written material on regulatory body website

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

REDACTED

Information will be made available as soon as it is ratified which can be within months of studies being completed and therefore over the five years significant data will be shared.

How will you maximise the outputs of your work?

Collaboration- liaison with other international and european groups to avoid duplication and maximise study output

Dissemination of new knowledge - through peer reviewed publication, presentation at international and european conferences - this would include any anomalous findings

Species and numbers of animals expected to be used

REDACTED

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

REDACTED

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

We expect our studies to have extremely low impact on our horses. Adverse effects are expected to be minor and transient.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The level of severity expected in these studies is mild. EDACTED. A more significant (anaphylactic) reaction could involve widespread (systemic) signs such as fever, limb or facial swelling. Anti- inflammatory medication and a specific protocol of veterinary intensive monitoring and care would be used to deal with this. REDACTED To reduce the number of times a needle is used to take a blood sample , REDACTED
<1% of horses used experience any adverse effects.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 14 July 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

REDACTED At this time , generation of these data cannot be satisfactorily modelled without the use of animals because metabolism of drugs can be very species specific. Although in clinical studies there has been some progress in translating ADME data from rodent species to other species (particularly man) it is also recognised that use of a second species (dogs and non-human primates) is often required to make the translation acceptable and only then because it is not considered ethical to use the target species (man) at this time.

REDACTED Nevertheless in parallell with *in vivo* studies , we continue to explore whether and how data can be extracted from our drug surveillance programmes, from published studies, (non-regulated samples) REDACTED.

What was your strategy for searching for non-animal alternatives?

There is on going research into ADME using *in vitro* equine hepatocytes systems using post mortem samples. This shows some comparison with *in vivo* studies but is not yet at a stage to replace *in vivo* studies.

Why were they not suitable?

REDACTED.

A retrospective assessment of replacement will be due by 14 July 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

REDACTED

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

We use statistical advice based on the close genetic makeup of the TB breed to minimise overall numbers. We take into account the Toutain method as mentioned above. We also conduct pilot studies to generate appropriate preliminary data to refine and reduce the overall number of horses required.

What other measures apart from good experimental design will you use to minimise numbers?

REDACTED

Re-use of the horses significantly reduces animal use .

A retrospective assessment of reduction will be due by 14 July 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

REDACTED

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

REDACTED

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

REDACTED

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We adhere to the ARRIVE guidelines.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We receive the NC3Rs newsletter and refer to the website. We consider the 3Rs for each individual study carried out and also review them at quarterly AWERB meetings.

Explain the choice of species and the related life stages

The purpose of this work is to produce information about how REDACTED; therefore it is deemed appropriate to use this REDACTED to obtain relevant, comparable data and valid results.

A retrospective assessment of refinement will be due by 14 July 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

14. Drug Delivery Platforms for Brain Disorders

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Insufficient translocation of drugs through the blood-brain barrier (BBB) has been widely recognised as a key component behind the failure of many treatments for brain disorders. Therefore, our aim is to understand the pathophysiological alterations to the BBB and take advantage of that to design novel delivery vehicles that would enhance drug translocation into the brain.

A retrospective assessment of these aims will be due by 16 December 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Lesioned brain disorders (such as stroke, dementia and brain tumours) represent a leading cause of death and disability worldwide yet have very limited therapeutic options available. In the case of thrombotic strokes, for example, recanalization through thrombolysis or intravascular therapy have limited applicability and only being feasible in a low percentage (~10%) of patients. There is no effective therapy available for haemorrhagic strokes. Likewise, no effective treatments currently exist for other cerebrovascular disorders and brain tumours. There is an urgent need, therefore, to bring new ideas and approaches to the field to gain a better understanding of the disease pathophysiology, identify novel targets and develop selective treatment approaches. Insufficient translocation of drugs through the blood-brain barrier (BBB) has been widely recognised as a key component behind the failure of many therapies for brain diseases. Our recent research and that of others has identified that the nature of the BBB is altered in several brain diseases, yet, the underlying cellular and molecular mechanisms and the benefit of that disruption for brain drug delivery remains poorly understood. Current, treatment strategies for brain diseases do not fully address those pathophysiological adaptation of BBB in their design. Thus, there is an urgent need for a smart design of advanced drug delivery systems that take advantage of BBB structural changes after brain disorders.

What outputs do you think you will see at the end of this project?

This programme of work will provide the novel mechanistic insight into the structural and functional alteration of the BBB and other peripheral immune organs following brain lesions. It will define the nature of central and systemic pathological alterations in the early phase after a neurological event and the long-term outcome. By intervening in key pathways (e.g. tight junction protein disassembly) we will be able to define the contribution of specific pathways (e.g. transcellular vs paracellular pathways) to achieve maximum drug translocation to brain damage in stroke and other neurological conditions. By defining these parameters we will identify the time frame for maximum drug delivery into the brain and help decide on potential novel therapeutic targets that could be translated into treatments for patients.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The immediate benefit from this project will be for basic science by producing world-leading and internationally recognised work that will be published in very high impact journals. Output from this project will be utilised by research field , including preclinical and clinical academics, as well as industrial partners. We will disseminate our results to the public through various outreach activities and also through our work with charities (e.g. The Stroke Association, British Heart Foundation) and patient organisations to inform them of the findings of our research. While Basic science will benefit in the short-term (1-3 years), clinical impact will take longer period to be achieved. The latter will likely be beyond the five-year duration of the programme of work.

How will you maximise the outputs of your work?

The research findings of this project will produce the first comprehensive evidence on the advantages of selective drug delivery to the brain and other peripheral immune organs affected following certain neurological conditions. If the project outcomes proved positive,

REDACTED and other translation awards to confirm the results in multi-centre preclinical trials in-line with the step-wise approach recommended by STAIR. This will inform if initiation of clinical study will be considered.

Species and numbers of animals expected to be used

- Mice: 1400
- Rats: 300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

During this project we will develop different models of brain disorders. These include; brain ischaemia by (temporally cutting blood supply to the brain), brain haemorrhage (by inducing a local bleeding in the brain), brain tumour models (e.g. glioblastoma) and Alzheimer's models. Some of these models are performed by surgical operation during which the animals will be fully anaesthetised and will receive drugs (analgesics) to minimise any pain.

In some cases, we will combine these models with other conditions such as infections, obesity or diabetes to mimic the health conditions in patients who have multiple illnesses.

In the case of brain tumour models and Alzheimer's models we will apply non-invasive ultrasound externally to include transient opening of the blood brain barrier and therefore, improve the penetration of therapeutic substances into the brain and enhance clearance of harmful molecules that accumulate in the brain and exacerbate the disease condition.

Following induction of these models, we will apply some behaviour tests that are routinely used to check any problem with movement or sensation and memory disorders, and these are very similar to what is usually seen in patients with cerebrovascular disorder.

Behavioural tests are not harmful to the animals and last only for few minutes. However, the effect of the model itself such as stroke may result in discomfort to the animal, but the severity kept to minimum to ensure no lasting harm occur. We will undertake all possible measures to reduce unnecessary suffering or pain experienced by the animals and to apply appropriate humane endpoints if an animal shows persistent adverse events.

At the end of the experiment animals will be killed by overdoes of anaesthesia. We will take blood, brain and other organs and tissues that we will analyse to meet the projects aims.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Related to induction of co-morbidity

Infection: Infection dose will be kept minimum so as to induce only mild infection with limited adverse event to the animals.

Metabolic syndrome: Feeding animals a high-calorie e.g. high fat (or carbohydrate) diet for several months will eventually lead to obesity, and along with this the possibility of developing secondary conditions such as insulin insensitivity (as seen in obese humans). The impact this has on the animals will be reduced by limiting the duration of the study (e.g. up to 4 months).

In situations with multiple co-morbidities in the same animal it is possible that the animal might show an enhanced phenotype compared to those with just a single co-morbidity. For example, obese mice may show a more pronounced response to infection, we will therefore modify doses, treatment schedules etc to avoid producing too severe a phenotype, though we do not expect this to happen in the majority of cases.

Related to surgical procedures: Heat loss during the procedure and shortly after will be minimised by the use of an under-body heating pad and/or insulating material as required. During recovery from anaesthesia animals will be observed closely until fully conscious, using appropriate materials to minimise heat loss. When consciousness has been fully regained, they will be returned to their home cage. Animals will receive analgesia and fluids before, during and after surgery.

related to focal brain ischaemia: Brain damage due to acute cerebral ischaemia is likely to be associated with behavioural effects on the animals similar to those seen clinically i.e. hemiplegia and muscle weakness of the face, reduction in sensory or vibratory sensation, contralateral paralysis or weakness, loss of appetite and loss of balance or orientation. However, these well-described behavioural changes are generally limited to the first day or two after induction of ischaemia, thereby allowing recovery of animals for long-term longitudinal studies to study functional outcome. The level of impairment, both clinically and in experimental models, is determined by the amount of brain damage, which can vary and be controlled depending on the method used to occlude the MCA. In many studies we will use distal occlusion of the MCA, which produces a modest cortical infarct and therefore only minor behavioural changes.

related to intracerebral haemorrhage: Haematoma volume is the key determinant of outcome and therefore the dose of collagenase or volume of blood injected will be at levels sufficient to induce moderate blood loads in the brain but with minimal effect on the animal. The behavioural changes observed on the animal are comparable to those seen in focal brain ischaemia such as hemiplegia and muscle weakness of the face, reduction in sensory or vibratory sensation, contralateral paralysis or weakness, loss of appetite and loss of balance or orientation (though not as potentially severe) and are generally limited to the first day or two after induction of intracerebral haemorrhage.

related to brain ultrasound and imaging: No adverse effects are expected apart from those associated rarely with general anaesthesia including death due to anaesthetic accident and dehydration. Appropriate action shall be taken to limit these in that body temperature will be monitored and maintained with a heating element, making hypothermia unlikely. Monitoring of pulse and/or respiration will also be performed to make sure an adequate level of anaesthesia is maintained while hydration will be achieved as required although, as scanning periods are relatively short, dehydration is unlikely.

related to brain tumour: the majority (~80%) are expected to grow at a steady rate and will have no significant impact on the animal's general well-being. There is a possible risk of cerebral oedema (~2%) and / or systemic infection (~5%), arising from the surgery. If either of these are seen, they may be identified due to a rapid onset and deterioration (i.e., within 3 days post-surgery) of the following clinical signs: pilo-erection, lethargy, ataxia and hunched posture. In the event, these signs are observed or weight loss of up to 15%, then the animal will be killed by S1 method.

Related to Alzheimer's model: age-related cognitive deficits may be observed such as spatial memory, cognitive deficits which are associated with anxiety, circadian rhythm, and aggression. These symptoms are age related and has been reported before to first observed at 3-6 months and increase with age up to 12-14 months and generally are not expected to produce any major effects on animal wellbeing. The age of the animal at the start of the experiment will be decided based on the experimental design and the scientific question to be answered. No animals will be kept beyond 15 months of age.

Related to behavioural testing

The majority of behavioural tests are painless, stress free and non-invasive and therefore not associated with any adverse effects. No adverse effects of the behavioural paradigms we plan to use are normally seen other than mild anxiety to a new environment which quickly disappears.

The majority of the studies will be done with short time point between few hours to 72h after induction of the models specified above. In some experiments long term functional studies will be performed over longer period of time (e.g 4 weeks) to study long term functional recovery after treatment with novel therapeutics. Clinically post-stroke complications can manifest some weeks after the event and persist for many years. We will therefore in few experiments may monitor animals for up to 15 months post- stroke, though most studies will be completed well within much shorter time.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

It is inevitable that a small proportion of animals will show adverse effects, due to the nature of the intervention. The majority of the animals will be performed in mice and only few experiments will be done in rats. From those 43% (of mice and rats) are expected to develop maximum moderate severity which include those used to induce intracerebral haemorrhage model and those tested with brain US. Animal used under Protocol 2 that involves brain damage due to acute cerebral ischaemia will experience a maximum severe severity limit. This constitute approximately 29% of the mice and rats used under this licence. The rest of the animals (28%) will experience either moderate severity limit (sham operated animals) or mild (control animals that do not undergo surgical interventions).

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 16 December 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Studying mechanisms involved in brain diseases such as stroke is extremely complex. Alongside the death of cells in the brains of stroke and dementia patients, these diseases are characterised by profound changes in behaviour, which it is not possible to study in cells in isolation. Moreover, rodents develop complications that are often observed in humans such as post-stroke infections.

What was your strategy for searching for non-animal alternatives?

The proposed animal studies are complementary to a broad programme of work on brain diseases using in vitro 2D and 3D cell culture models, clinical samples and non protective species.

Throughout the project we will seek, review and incorporate alternative methods based NC3Rs recommendations.

Why were they not suitable?

A key point in this project is the need to study the changes to the blood brain barrier after brain diseases. Studying the causes and consequences of blood brain barrier disruption is a challenging research area that requires an understanding of the complex interactions between multiple physiological systems i.e. the nervous and immune systems. Currently, it is not feasible to mimic these complex communications ex vivo, as there are no in vitro models that can replicate the complex structure of the brain and its connection to the immune system. Therefore, our objectives cannot be fully studied in cell culture model alone and thus whole animal in vivo experimentation becomes unavoidable to gain a full understanding of the mechanisms involved.

A retrospective assessment of replacement will be due by 16 December 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise

numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have used data from preliminary experiments and previous studies to estimate the means and standard deviation (SD) for key primary and secondary outcomes. We will then perform power calculations for these outcomes to calculate the sample size necessary to detect a difference of at least 20-40% assuming a significance level of 0.05 and power of 80%. Exclusions due to surgical complications and mortality occur in up to 30% of the mice, therefore we correct for that in our final sample sizes.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

We will ensure that we use the minimum number of animals required to answer the scientific question by performing power calculation studies. We will also apply the NC3Rs experimental design assistant tool for appropriate experimental planning. We will regularly consult qualified statisticians about experimental design and statistical analysis.

What other measures apart from good experimental design will you use to minimise numbers?

We will use the NC3Rs experimental design assistant (EDA) tool for appropriate experimental planning. All experiments using live animals will adhere to the ARRIVE guidelines on design and reporting. Good principles of experimental design will be applied to ensure sufficient group sizes will be used to adequately test the hypothesis. Sample sizes are estimated from pilot studies and previous data using power analysis. Animals will be randomly allocated to the experimental conditions.

Treatments will be administered in a blinded manner. Mice and subsequent tissue and blood samples will be labelled in such a way that the of the data analysis and outcome measures will be blinded to the allocation of experimental group.

A retrospective assessment of reduction will be due by 16 December 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Rodents have brain structure comparable to humans and the location of injury and mechanisms of brain damage are also comparable. Moreover, mice develop complications like those seen clinically e.g. spontaneous infections and behavioural changes that are very relevant to study the project.

We will use previously published models of cerebrovascular disease such as ischaemic and hemorrhagic models of stroke, orthotopic brain tumour models and Alzheimer's models that are very well established and widely used in many laboratories throughout the world. The choice of the model will largely depend on the hypothesis being tested. Although there is no ‘perfect’ model for the conditions we will test in this project, the models selected for this project are based on the pathological and behavioural similarities to cerebrovascular disease in humans, which itself is extremely heterogeneous.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The studies proposed in this project could not be undertaken in animals with a lower degree of neurophysiological sensitivity (e.g. drosophila, C.elegans) because those models don't show comparable complications seen in humans. Similarly, in vitro experiments would not permit testing the interaction between different body systems.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

All animals will be very closely monitored for any unwanted effects. We will follow the very recent guidelines published by the stroke community in collaboration with NC3Rs to minimise these.

Examples on that include; enhanced monitoring during the critical 24h period after stroke surgery, use of analgesia, use of aseptic technique and post-operative care refinements. We will ensure that initial animal health is established and will be maintained to minimise any risk of infection through environment management and applying principles of biosecurity barrier. Whenever required environmental enrichment strategies will be considered and advice will be taken from NVS/NACWO as appropriate.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

In all our procedure we will refer to published guidance (e.g. IMPROVE guidelines, Workman guidelines) to make sure we apply the best current practice starting from the correct handling methods and applying the minimal invasive techniques in choosing the route of administration, volume size, administration frequency and needle sizes to induce the least harm possible to the animals. We will apply repeated animal observations and objective measurements to identify and define any adverse effects and to ensure humane endpoints are applied to minimise any harms. We will ensure that our team are well trained and have the expertise and experience required to perform the work at high standards.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Throughout the project we will continually review the literature and engage with colleagues/collaborators to learn of any new refinements to the protocols that could be implemented. We will also adhere to the ‘The IMPROVE Guidelines that are published recently in the Journal of Cerebral Blood Flow by NC3Rs working group on stroke.

Explain the choice of species and the related life stages

Most of our experimental procedures will be done in mice and in some experiments will use rats. Rodents have similar cerebral and cerebrovascular anatomy to humans and the location of injury and mechanisms of brain damage are also comparable. Moreover, mice develop post-stroke complications like those seen clinically e.g. spontaneous infections and behavioural changes that are very relevant to study the objectives of this project. Factors such as age and sex are well-known to impact the outcomes of the study. Therefore, at this stage of our project we will reduce unwanted variabilities by conducting our studies in adult male rodents to improve signal to noise ratio and increase the power of the experiments.

A retrospective assessment of refinement will be due by 16 December 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

15. Evaluation of fish vaccine potency and interaction with other disease control factors

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)
- Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

No answer provided

Animal types	Life stages
rainbow trout	juvenile
Zebra fish	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

To evaluate potency of established fish vaccines against vibriosis, photobacteriosis and enteric redmouth (ERM), as well as to discover, develop and test methods for prevention of fish diseases important to the aquaculture industry.

A retrospective assessment of these aims will be due by 24 December 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Fish disease is the main constraint on the expansion of aquaculture. Since wild fish production is not increasing, but human population is, aquaculture must increase to maintain global food security in the coming decades. Prevention and control of fish diseases are critical to allowing the aquaculture expansion necessary to feed the human population in future. Development of disease prevention measures such as vaccines and in-feed immunostimulants reduces the need for chemical and antibiotic treatments, which in turn reduces release of these harmful compounds to the environment. They also reduce disease incidence in farm facilities, so reducing the opportunity for transfer to wild fish populations. These effects aid protection of the natural environment.

What outputs do you think you will see at the end of this project?

It is anticipated that 50 - 100 batches of vaccines will be released to market as a result of work under this licence application

New information: increased insight into virulence and control of Vibriosis, which will be disseminated through peer-review publications. Refinement of vaccination procedures through improvement of oral vaccine efficacy and expansion of orally administered vaccines.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Aquaculture industry will benefit immediately vaccines are successfully tested (potentially within 2 months of start of project).

European consumers will benefit from availability of food fish supply.

Research community will benefit from new information disseminated through peer reviewed publications.

How will you maximise the outputs of your work?

Dissemination of new information through publications, meetings of learned societies and local knowledge exchange groups

Publication of evaluation of testing regimes and changes in characteristics of pathogenic bacteria

Collaboration with industry partners and Innovation Centres to maximise the communication and uptake of new findings.

Species and numbers of animals expected to be used

- Other fish: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

A typical animal will be immunized (one of three routes - injection; immersion in vaccine solution or administration of vaccine in food), before a holding period (4 weeks) to allow immunity to develop. After this, the fish would be injected in the abdominal cavity with 0.1 ml of bacterial pathogen. The fish would then be held for 3 weeks to check for development of disease (if the vaccine is effective, little or no disease signs should be evident; sham vaccinated controls will develop disease). At the end of this period, all remaining fish would be euthenised. Thus, most fish would experience 2 procedures (immunization and pathogen injection).

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Impacts as a result of disease development can include abnormal behaviour over a period of 0.5 - 1.5 hours.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Severe - 33 %
Moderate - 5 %
Mild - 62 %

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 24 December 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Potency testing of fish vaccines requires in vivo testing as stipulated in the European Pharmacopoeia 10th edn. (2019), overseen in the UK by the Veterinary Medicines Directorate. At present no alternatives are available, as fish immunity is largely dependent on cellular immunity, which is multifacetted and for which there is no available proxy or in vitro assay (unlike antibody production which can be used in mammals over a short timescale). Antibodies are produced in fish, but often beyond the timescale required for vaccine testing (1-3 months). Longer potency tests are not practical owing to constraints on vaccine shelf-life and the likelihood of natural fish mortality over longer timescales, which make a shorter, whole animal-based test most appropriate.

Investigating interaction of feed additives and vaccines with the immune system requires animal use, as the physiological systems involved are too complex to replicate in vitro at present.

What was your strategy for searching for non-animal alternatives?

Isolated organ culture (such as gills) and cell culture, which may provide alternatives to in vivo testing in the future, but at present are insufficiently well developed or characterised to replicate the complex interactions contributing to an efective immune response in vivo. Consequently, such methods do not yet reach the level of rigour necessary for regulatory approvals.

Consequently, vaccine potency testing needs to continue using whole animal tests at the moment, as this is the only way currently available of encapsulating the complex interactions of the vaccine with the fish immune system and the consequent development of effective immunity.

Why were they not suitable?

Isolated organs cannot be kept viable long enough to investigate disease effect, and do not replicate interactions between organ systems.

Cell cultures only represent single cell types and cannot replicate organ system interactions as seen in whole animals.

A retrospective assessment of replacement will be due by 24 December 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise

numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Trout numbers estimated from numbers used in previous years' testing - group sizes (20-30 per group; 5-10 for controls) specified in regulatory protocols (European Pharmacopoeia, (2019) 10th edn.).

Salmon - power analysis indicates sample sizes of 16-20 per group for effect sizes of ~10-15% (e.g. cell counts)

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Online tools, including power analysis calculator and NC3Rs experimental design assistant. Vaccine work determined by regulatory requirements

What other measures apart from good experimental design will you use to minimise numbers?

Use of pilot studies to explore effect sizes and to ensure appropriate disease challenge doses. Sharing of tissue samples across projects to maximise analysis and output. Sharing control groups between batches to minimise control (unvaccinated) numbers.

A retrospective assessment of reduction will be due by 24 December 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

In studying disease, it is necessary to induce disease. The methods are aimed at combating disease, so the treatments (vaccines, additives) should reduce suffering and distress. In order to evaluate these treatments effectively it is necessary to have untreated controls which develop disease. These are monitored to allow as rapid intervention as possible with onset of disease symptoms, to reduce suffering as much as possible.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Vaccine testing regulations specify the stage and size of animals to be used, which cannot be varied. For characterisation of disease and analysis of factors affecting this, the interplay of organ systems in vivo is crucial to understanding the disease, and using less sentient individuals would not allow relevant life stages to be used (in the case of more immature stages) or long enough maintenence to see disease progression (in the case of anaesthetised animals).

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

The intended end-point is morbidity (development of disease signs) with intervention and euthenasia before death. Onset of disease is usually recognised by altered equilibrium (uneven swimming) and discoloration of the skin. When these signs are observed, fish will be euthenised using a Schedule 1 method. To achieve the intended end-point, increased monitoring (3 to 6 hours, depending on the pathogen; more frequent monitoring risks accelerating disease progression owing to increased disturbance of the animals) will be performed during critical stages of disease development (usually 2 - 6 days post infection). Observed decrease in morbidity rate will be used to evaluate when increased monitoring can be relaxed, on a test-by-test basis, given inherent variability of the interaction between individual pathogen cultures and different stocks of fish.

Anaesthesia (pain management) will be used to avoid additional stress or injury during handling and injection.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

NC3Rs guidance

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Regular survey of relevant peer review journals.
Regular review of the NC3Rs website. 6 monthly meeting with NACWO on fish welfare issues and implementation plans.

Explain the choice of species and the related life stages

For vaccine testing, the types and life stages used are specified in regulatory protocols as being the most relevant to commercial application of the vaccines.

For basic research the types and sizes of animals used are determined by relevance to future application of the findings.

A retrospective assessment of refinement will be due by 24 December 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?

- During the project, how did you minimise harm to the animals?

16. Evolutionary ecology of malaria parasites

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

No answer provided

Animal types	Life stages
Mice	adult, neonate, embryo, juvenile, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

To understand the strategies that parasites have evolved to cope with the challenges of their lifestyle and to exploit the opportunities it brings. To identify the evolutionary limits on how parasites maximize their fitness, in terms of their ability to survive in the host they infect and to transmit between hosts. Our work involves assessing how parasites interact with each other and respond to the changing environments they experience inside hosts and the vectors, such as mosquitoes, that transmit them.

A retrospective assessment of these aims will be due by 01 November 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Malaria parasites and their Apicomplexan relatives are responsible for some of the most serious diseases of humans (e.g., malaria), livestock (e.g., coccidiosis), companion animals (e.g., toxoplasmosis) and wildlife (e.g., avian malaria). For example, almost half the world's population are at risk from malaria; it kills approx. half a million people each year, mostly children under 5, and several hundred million cases occur annually. Resistance to all antimalarial drugs has evolved and no vaccine has been licensed for widespread use. New interventions are needed as well as insight into strategies that make the best use of existing tools. Therefore, our research and results are relevant to applied medical and veterinary science.

More broadly, some of the biggest problems facing disease control are a consequence of evolution. Explaining the evolutionary trajectories of hosts and parasites and how they interact to determine infection outcomes and patterns of spread are challenging but central to developing sustainable medical and veterinary interventions. Demonstrating that evolutionary theory can explain the traits, behaviours and strategies involved in host-parasite-vector interactions will inform the development of new drugs and vaccines as well as deployment policies.

What outputs do you think you will see at the end of this project?

Our research focuses on malaria (Plasmodium) parasites, which cause some of the most serious infectious diseases of humans, livestock, companion animals and wildlife all over the world. These parasites remain ahead of biomedical science, despite extensive research into their immunology, cell and molecular biology. Few new treatments or control measures have been discovered in the last decade. A different approach, using a whole-organism (evolutionary) perspective to understand how parasites behave has been neglected but is increasingly being recognised as central to advance disease control. Using an evolutionary approach provides a unique opportunity to understand parasite behaviours at all levels - from genes, to behaviour, to population patterns.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The scientific community benefits from my expertise in the evolution and ecology of parasites, which is internationally recognized. This is demonstrated by continuous applications from British, European, and North American candidates to join the lab, and >10 invitations per year to give seminars or keynote talks at conferences and in departments focused on evolutionary biology and ecology, or medicine and parasitology, or chronobiology, and by the >20 publications stemming from work on the current PPL (despite maternity leave and part time working).

My lab is also very active in public engagement. Our focus is developing and deploying activities for children of all ages, including a dedicated grant to carry out school visits and regular (3x year) stands at open days and science festivals.

Malaria parasites have high medical importance and the development of new control strategies requires a better understanding of their evolutionary biology. We will contribute to this understanding and the development of control/treatment strategies.

How will you maximise the outputs of your work?

My lab collaborates widely (e.g. with researchers in the UK, North America, Canada, Saudi Arabia, several European countries). We publish findings as soon as possible in Open Access journals and also post to pre-print servers as soon as manuscripts are ready for submission. Our publications are often the topic of articles in the lay press and attract diverse media attention. In addition, I am integrating evolutionary thinking into parasitology and medicine through international interdisciplinary consortia and symposia that set the directions for emerging topics in evolutionary parasitology and chronobiology.

Species and numbers of animals expected to be used

- Mice: Total = 10000 [P1 = 4000; P2 = 3000; P3 = 2000; P4 = 1000]

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Our experiments require monitoring parasites and hosts during infections and examining parasite behaviours, physiology and genetics. Infected animals are monitored at least once a day (but often round-the-clock occurs) and in some experiments, they do not show any clinical symptoms. When infections need to be monitored throughout their natural course, animals experience weight loss and anaemia, and almost all recover fully. We euthanize any that are at risk of not making a full recovery. We take small blood samples to collect our data and to monitor the health of animals. Mosquitoes feed on mice that have been anaesthetised and they only take very small quantities of blood. All animals are euthanized at the end of experiments.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Infected mice in some experiments will not show many clinical symptoms beyond mild piloerection.

In experiments where parasites are monitored throughout infections, mice can become inactive and anorexic for 2-4 days, experiencing weight loss and anaemia at the peak of infections. After this time, >90% mice control their infections and fully recover.

Mice are sampled daily from their tail vein when <20ul blood is usually collected for red blood cell counts and quantification of parasite stages and to monitor the health of animals.

For mosquito blood feeds, mice are lightly anaesthetised and exposure only lasts for 30-60 minutes.

Parasites and most drugs are administered using IP or IV injections. ID tags are administered subcutaneously.

All mice are under general anaesthetic when large numbers of parasites are collected.

No infection results from using mice to maintain the mosquito colony and anaesthesia is always given.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Mild 57.5%, includes animals used for:

Maintaining mosquito colony

Short term infections

Generating parasite material for culture/preservation

Behavioural experiments using uninfected mice
Breeding colony for GA strain

Moderate 30%, includes animals used for:

Infections terminated at the end of the growth phase of infections, before severe anaemia/anorexia occur

Entire-infection experiments with avirulent parasite strains/species

Experiments in which interventions (e.g. antimalarials) are used that reduce parasite replication

Severe 12.5%, includes animals used for:

Entire-infection experiments with virulent parasite strains/species

Experiments with novel combinations of host/parasite strains or perturbations

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 01 November 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

To understand how parasites and hosts interact and their co-evolution, both parties are required. Ethically, it is not appropriate to use human malaria. Therefore, experiments are only possible with animal models. In vitro methods cannot capture the complexity and biological context of real infections.

What was your strategy for searching for non-animal alternatives?

We employ mathematical modeling approaches where possible to form and refine hypotheses but ultimately, we need to use animals to formally test our scientific hypotheses. We continually harness new technical advances from the fields of evolutionary, molecular and cell biology and immunology to reduce numbers. We have been instrumental in developing some of the in vitro methods we use. We can now reliably culture several parasite strains throughout a single development cycle (24 hours), but longer-term culture eludes us because it has proved impossible to achieve sufficient re-invasion of RBC of the next developmental cycle. Other developments include the discovery that replenishing media post fertilization significantly enhances yields of parasite offspring.

We also use the most powerful statistical analysis methods available for data analysis as this maximises the amount and quality of information obtained from each animal. We use our data to answer multiple research questions, reducing the need for multiple experiments.

Parasites are stored in liquid nitrogen, reducing the number of animals needed to maintain them.

Why were they not suitable?

In vitro methods cannot be used for the entire parasite lifecycle. Neither culture or mathematical models can capture the complexity and ecological context of real infections.

A retrospective assessment of replacement will be due by 01 November 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Animal numbers are estimated based on our current usage, and the anticipated number of researchers involved.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

With the substantial effort and expense of the experiments, developments to improve science quality and reduce animal usage are simultaneously achieved. For example, we:

Have developed in vitro methods to estimate the mating success of several malaria parasite species, reducing the need to blood-feed mosquitoes on infected animals.
Have developed in vitro methods to culture asexual parasites throughout a cell cycle. This enables us to produce large numbers of mature parasites from fewer mice and to examine cell cycle transitions.

Have established membrane feeding for mosquitoes, reducing the need to expose animals to mosquito bites.

Use data from each experiment up to answer multiple research questions, reducing the need for multiple experiments.

Parasites are stored in liquid nitrogen, which saves continually maintaining them in mice and prevents virulence from increasing.

Compared to the current PPL, we are able to reduce animal numbers by >40%, and entirely remove 1 species.

What other measures apart from good experimental design will you use to minimise numbers?

We primarily use the excess generated from breeding colonies to maintain our mosquito colony, minimising the number of animals specifically bred for this purpose.

We use both male and female mice in our experiments, and do not restrict usage to a narrow age range. This ensures no excess animals are produced by our breeding colony and minimises the excess in the colonies of the suppliers of the other REDACTED we use.

We closely monitor the production of our mouse breeding colony, moving animals from breeding to experiments efficiently and contracting the colony whenever possible. Likewise, we run a skeleton mosquito colony whenever possible which reduces animal usage by 50% compared to that required to produce mosquitoes for experiments.

A retrospective assessment of reduction will be due by 01 November 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Our research involves malaria parasites that infect rodents in nature. Our experiments require monitoring parasites and hosts during infections and examining host and parasite behaviours, physiology and genetics. We infect animals either from mosquito bites or by injecting parasites. We then take small blood samples to collect our data and to monitor the health of animals throughout infections. Some animals are not sampled but are given RFID tags to monitor their activity patterns and body temperature without disturbance. When large numbers of parasites are collected, animals are under terminal anaesthesia. Our procedures for inducing perturbations and sampling are generally expected to cause no more than transient discomfort and/or slight changes in behavior.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

By definition, the study of the ecology of infections requires allowing infections to play out in as realistic a context as possible. Infections are usually studied for between 5-15 days and so, animals cannot be under sedation for so long.

Malaria parasites only infect terrestrial vertebrates and so, less sentient species are not suitable hosts.

Infection of juvenile animals would result in far more severe infections and the smaller blood volume of juveniles would reduce the amount of information available from samples.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Over the last 15 years our work has been refined. For example, the majority of animals will be euthanized before showing clinical disease symptoms, less virulent parasite strains feature more frequently in our experiments, we have improved the ways that we administer drugs and blood sample infected animals, and we avoid single-housing mice so they can benefit from huddling and social interactions.

We are continually refining our endpoints as we gain more experience with different host-parasite strain/species combinations and experimental perturbations. Infected animals are monitored at least daily and often around-the-clock during symptomatic periods, so that measures can be taken to facilitate their recovery. If animals show signs of sickness they are given supportive nursing measures (e.g. mash, transgel). We are also investigating whether surface body temperature is an informative endpoint and whether gentle warmth from heatpads is another useful supportive nursing measure.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Local guidelines for blood sampling.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

The REDACTED communicates advances and development opportunities regularly. During the current PPL we have implemented procedures to communicate more closely with animal care technicians to inform the monitoring of animals and how to make interventions more effective. We will continue to foster these lines of communication.

Explain the choice of species and the related life stages

Mice are used as hosts for experimental malaria infections to study infection dynamics, parasite phenotypes (e.g. developmental rhythms), and host responses to infection. Mice are also used to maintain a colony of mosquitoes for studying parasite transmission and maintaining the wild type phenotype of parasite isolates.

To understand how parasites and hosts interact, both parties are required. The aims cannot be met using field-based research with human malaria infections. For ethical reasons, it is not possible to have the necessary untreated control groups, provide sub-curative drug treatment, or prolong infections for research purposes. We use in vitro methods where possible (for some estimates of transmission potential, generation of parasites for transfection, and some cell cycle studies), but in vitro methods cannot capture the complexity and biological context of real infections.

Rodent malarias are an excellent model for human malarias and have been successfully used in lab experiments for over 50years. Much of what is known about the genetics, cell and molecular biology of malaria parasites, the evolution of anti-malarial drug resistance, and immune defence against malaria come from the extensive body of work using rodent malaria model systems. This has been achieved thanks to the uniquely well-characterized biology (e.g. immunity and physiology) of lab mice. Murine immune responses and their circadian clocks are well defined and the technology enabling sophisticated manipulations of the haematopoietic and immune system is highly developed.

A retrospective assessment of refinement will be due by 01 November 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

17. Fish behaviour modification through gear changes

Project duration

5 years 0 months

Project purpose

None selected

Key words

Fish Behaviour, Selectivity, Artificial Light, Gear Modifications

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The overall aim of the project is to investigate how fish behaviour can be used to make commercial fishing gears more selective through controlled tank experiments. Selectivity of fishing gear is particularly important in fisheries with a mix of species such as the North Sea. Therefore it is vital fishermen are selective in what they catch and only retain fish of the correct species and size, particularly with the landing obligation now fully implemented meaning fishing vessels can no longer discard unwanted catch back to the sea but must land everything that they catch. A variety of gear components with a range of modifications made to them, such as visual stimulus's like artificial light, different coloured twine etc., will be positioned in the tank to simulate a commercial gear in a series of controlled tank experiments. This is to enable us to get an understanding of how fish will respond to the modifications to the gear and whether any behaviours identified could be utilised in encouraging escape and avoidance behaviour during commercial fishing to improve selectivity. The first gear modification we will be investigating is the use of lights (either laser powered fibre optic cables or LEDs) to encourage or deter fish to pass through the meshes of the gear. If differences in how certain species behave towards the lights or any of the gear modifications tested are found we could potentially use this to increase the selectivity of the gear and target particular species while allowing unwanted species to escape by re-directing them to an escape hole in a different section of the gear. Gear modifications could also potentially encourage the escape of undersized fish while retaining the larger marketable catch by making escape routes more visible with lights and this aspect of the gear modifications will also be investigated. Further work will observe the effects of other gear modifications such as sound, different coloured twine etc. on a range of commercially important fish species.

A retrospective assessment of these aims will be due by 05 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The potential benefits of this work are that it may assist commercial fishing vessels to fish in a more sustainable and environmentally friendly way. It could potentially help to reduce the capture of undersized fish or avoid the capture of unwanted species which would nicely compliment the landing obligation scheme which was fully implemented as of 2019. It could also exploit species specific behaviours to enhance the selectivity of trawls which is particularly useful in a mixed fishery as the North Sea.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We will be using a range of marine fish throughout the work. Approximatley 1500 over 5 years

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

The expected adverse effects will come from the investigations into escape behaviours of the fish when chased or encouraged towards the gear component which may cause moderate stress. During the escape behaviour work a single experiment will consist of the fish only having to swim one length of the seven metre length of the tank two times a day. A set number of trials will be decided through discussions with a statistical consultant and adequate respite between trials will be given to counteract the moderate stress for the short time period experienced by the fish during a single trial. Certain trials will use laser pods to power long lengths of fibre optic cables, the lasers used are class 3B and can be harmful to the naked eye when viewed. All suitable precautions are in place to ensure both personnel working with the lasers and fish are not at risk of viewing the laser beam and the only part of the equipment the fish will see is the fibre optic cable. This includes the set-up of the laser pods out with the experimental tank and locking the fibre optic cable to the laser pod prior to power being supplied means the likely-hood of ever viewing the laser beam is highly unlikely so risk is minimal.

A retrospective assessment of these predicted harms will be due by 05 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

As we will be observing fish behaviour we have to use actual fish to learn what their responses will be to the gear modifications. A computer model would require prior knowledge of how the fish will react and is therefore not currently an option for our study but we will use the data to develop one in the long term if possible.

A retrospective assessment of replacement will be due by 05 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

To minimise the number of fish used throughout our experiments we will carefully plan our experimental design to ensure it is statistically valid with assistance from our statistical consultant. We will ensure we have enough fish to make reasonable conclusions from our work and have enough power to detect any changes when carrying out the statistical analysis of the results. We will also be carrying out small scale observational studies to understand a range of marine fish species reactions to light or sound etc.
which will provide us with an insight into how to proceed with the full scale trials involving the gear components towed or moved within the tank.

A retrospective assessment of reduction will be due by 05 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We will be using marine species of commercial interest to help us meet the aims and objectives of this project. It is crucial we use these species because understanding their behaviour is key to the success of this work and to potentially improving the sustainability of these commercially important fish stocks. We need to replicate the stresses and pressures exerted on the fish that they experience during the commercial capture process to enable us to draw reasonable conclusions from the work. By starting with small scale observational trials (~6-8 fish) we will optimise the design of the study to achieve our aims in a timely manner with the least amount of animals being used as possible. We will be using voluntary behaviours or the use of a food reward which is positive non-painful encouragement for the small scale trials However it is known that during commercial fishing fish are stressed and under pressure to make a decision whether to attempt to escape through the gear, hence we will need to exert some level of stress upon the fish to mimic this effect to evoke a reaction in our fish when we proceed to the full scale trials (10+ fish). All fish will be monitored throughout the process and limited to a number of times where they will be used within an experiment with adequate respite in-between.

A retrospective assessment of refinement will be due by 05 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

18. Gene Therapy for Lung, Liver and Other Disorders

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

To evaluate the efficiency, duration and efficacy of transgene expression mediated by a variety of gene transfer and gene editing approaches for the treatment of a range of lung, metabolic and systemic diseases.

A retrospective assessment of these aims will be due by 07 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The targeted human diseases are associated with high mortality and/or a high burden of care (daily physiotherapy sessions for certain lung diseases, frequent injections for other diseases) and considerable costs to the NHS (ranging between £15K and £500K per patient). A potent gene therapy for any of these diseases will provide a significant improvement in the quality of life of affected individuals and a reduction in NHS costs.

What outputs do you think you will see at the end of this project?

We anticipate publishing multiple scientific publications as a result of the studies performed under this PPL.

It is hoped that as a result of the studies performed under this PPL, new drugs will eventually become available to treat these and related disease. A major step in making a new drug available, is performing clinical trials - where drugs are tested in small groups of human volunteers. The PPL applicant has a strong track record in translating ideas evaluated in animal studies to clinical trials in patients; and it is anticipated that further clinical trials will result from the studies conducted under this PPL.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

In the short-term (simplistically - throughout the duration of the PPL), scientific publications will benefit scientists working both generally in gene therapy and specifically in the disease areas gene; and, medical doctors looking after patients with CF, chILD, A1AT-deficiency and WD.

In the medium term (simplistically - towards the end of the PPL and within a few years of its completion), conducting early-phase clinical trials tends not to benefit those generous enough to volunteer to be involved, as such studies often focus on drug safety rather than drug efficacy. However, volunteers on late-phase clinical trials (especially those involving long-acting gene therapy drugs) do often experience life-changing improvements in disease symptoms and/or progression.

In the long-term (simplistically - ongoing over next 10-20 years), making new drugs available to treat these disease will impact both the lives of those suffering from them and, the other individuals who support them (e.g. parents, partners, carers). It is anticipated that the developed drugs will ultimately decrease the frequency of engagement with the healthcare system and overall result in a decrease of healthcare costs - improving the health and welfare of society at large.

How will you maximise the outputs of your work?

The applicant is one of the founding members of both the REDACTED. Advances made under this PPL are immediately available to a group of ~50 scientists working on these projects in three UK Universities.

Information sharing happens via weekly telephone calls and an extensive schedule of review meetings. This pooling of scientific results and best practise Informing complimentary in vivo research at these sites conducted under separate PPLs.

Both of these scientific consortium have public engagement activities - host laboratory visits and encouraging outreach activities; including: public lectures (often to groups with an interest in specific diseases) and visits to primary and secondary schools.

Species and numbers of animals expected to be used

- Mice: 14,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

The purpose of the PPL is to investigate the use of gene therapy and gene editing as potential new treatments for a range of serious genetic (CF, chILD, A1AT-deficiency, WD) disorders. Over the course of the PPL we are aiming to improve of our gene therapy approaches and thus are typically attempting to develop formulations with increased efficiency and duration of effect.

For the majority of studies we will administer novel gene therapy formulations (e.g. new mixtures of DNA and lipids and/or polymers or safe version of a common virus) to the nose and/or lungs of mice. In a minority of studies, the same sorts of formulations are administered via an injection into to either a vein, the leg muscle, the peritoneum or under the skin. For most of these administration routes we anaesthetise the animal.

After gene therapy formulation delivery, we typically ask up to three research questions - how efficient was gene delivery, how long does gene expression last and can the gene delivery approach under evaluation correct a physiologic defect associated with one of our target disease. Thus, after gene delivery animals are allowed to recover for a variable period (days to months) after which time we assess transgene expression and/or a physiologic parameter. One common way we measure transgene expression is to use in vivo imaging - where, after anaesthesia, we inject a molecule that can be modified to emit light and then place the animals in a chamber under a highly sensitive camera. The amount of light emitted is a good measure of how efficient the gene therapy formulation under investigation was. In such studies we often measure light output several times with gaps (days to months) between measurements so as to understand the duration of gene expression.

The PPL allows considerable flexibility in the number of gene therapy formulation delivery events, the number of in vivo imaging events and the timing between them. However, the maximum number of general anaesthetic events allowed is ten - as typically one general anaesthetic events is needed for each gene delivery or in vivo imaging session, this also limits the combined number of such events to a maximum of ten.

To assess correction of a physiologic defect, we typically perform the measurements directly on terminally anaesthetised animals or kill the animals by a humane method and measure the physiologic defect in a sampled fluid or tissue using a laboratory test.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Gene therapy formulation delivery and/or in vivo imaging uses conventional administration routes and optionally general anaesthesia that in themselves induce no more than momentary pain or discomfort.

Administration via the nasal route can cause increased respiratory effort or cough but this resolves within a few minutes.

After gene therapy formulation delivery some animals can experience inflammatory responses to the gene therapy formulations - this typically results in "flu-like" symptoms that spontaneously resolve over 1-2 days. Symptoms include reduced movement and lack of grooming.

Several genetically altered animal models are expected to be used:

For the chosen CF, Sftpc, Abca3 an Serpina1 models appear healthy and thrive, however, there are some mild chronic respiratory issues - largely only measurable using sophisticated lung function tests that are conducted under terminal anaesthesia; the chronic nature of these issues dictates that the overall severity experience of such animals is moderate.

The chosen WD model appears healthy and thrives, however, there are some chronic mild liver issues and cognitive issues that relate to a chronic imbalance in copper levels; the chronic nature of these issues dictates that the overall severity experience of such animals is moderate.

For the chosen Sftpb model, there is a failure of the lungs to stay properly inflated - causing severe respiratory issues which if untreated can result in death.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The severity varies across the protocols to be used.

For Protocol 1: Breeding And Maintenance Of GA Animals (5000 animals), 20% are expected to experience no more than mild harm, while 80% are expected to experience moderate harm.

For Protocol 2: Breeding And Maintenance Of Sftpb GA Animals (2000 animals), 80% are anticipated to experience no more than mild harm, while 20% are anticipated to experience moderate harm.

For Protocol 3: In Vivo Gene Delivery (6000 animals), 80% are anticipated to experience no more than mild harm, while 20% are anticipated to experience moderate harm.

For Protocol 4: Sftpb Rescue (1000 animals), 25% are anticipated to experience no more than mild harm, 50% are anticipate to experience moderate harm , while 25% are anticipated to experience severe harm.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 07 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Where possible we use cell culture experiments to evaluate our gene therapy formulations that perform gene transfer or gene editing. However, no cell culture models currently available recreates all aspects of the interaction between the organs we are trying to treat and the gene therapy approaches we are using. In particular, these non animal approaches lack an immune system, which we and others have shown is crucial for understanding how gene therapy might work when tried in humans.

What was your strategy for searching for non-animal alternatives?

We have used portions of human and animal lung, muscle and liver to study gene therapy formulations in the laboratory. Some of these studies have been very helpful as they have allowed us to replace some animal studies we might otherwise have performed with laboratory studies.

One approach is culturing human lung cells in a way that ensure they have air on one side and liquid on the other. This is more similar to the human lung than traditional cell culture methods. We call this approach ALI - short for culturing cells a the air-liquid interface.

A second approach, called ex vivo lung perfusion (EVLP), uses excess donor human lungs that aren't suitable as transplant tissue. We culture these lungs using an oxygenated blood substitute.

A third approach, called precision cut lung slices (PCLS), allows excess human donor tissue or tissue we obtain from other animals to be cultured for long periods of time.

A fourth approach is the creation of mini lungs and mini livers in the test tube (these are often termed organoids) by sophisticated cell culture approaches.

Why were they not suitable?

ALI studies have been highly informative and we routinely use these prior to initiating animal studies.

EVLP studies have been difficult for two reasons: (i) the donor tissue is in high demand and thus we have not been able to perform many studies, and (ii) the tissue is difficult to keep alive.

The PCLS approach has proved useful - particularly at the end of EVLP experiments where we can use this approach to extend the duration of experiments in a way that is just not possible with EVLP itself.

The organoid approach is new to our laboratory but looks a very encouraging approach to reducing the numbers of animal studies we perform.

Crucially, none of these replacement options recreates all aspects of the interaction between the organs we are trying to treat and the gene therapy approaches we are using. In particular, these non animal approaches lack an immune system, which we and others have shown is crucial for understanding how gene therapy might work when tried in humans.

A retrospective assessment of replacement will be due by 07 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We routinely use power calculations to support group size selection. As we typically have large effect sizes, group sizes are typically 10 animals or fewer.

To estimate the numbers of animals used on this PPL we have used historical values from REDACTEDs, and an assessment of the number of studies needed to deliver the project plan to build a mathematical model. For example, one protocol on this PPL is used to support five scientific projects over five years, we anticipate performing 10 studies per year for each project and that each study will include three groups of eight animals. Combining these estimates, allows us to calculate that we may use 6000 animals on this protocol over the duration of the PPL. (5 projects x 5 years x 10 studies per year x 3 groups per study x 8 animals per group = 6000).

We have applied this approach to each of the protocols to make the overall estimate.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

The key factor to controlling the number of animals used in this PPL is the group size selected for each study. In the worked example described above, reducing the group size from 10 to 8 results in a total reduction in the numbers of animals used in that protocol by 2,500, when applied to the entire PPL this reduces the estimate of animals used by ~3,500. Thus, the way we estimate group size is of considerable importance.

We use several tools to help estimate group size: G*Power is an open-source, free to use, statistical software package that we have used over many years to help estimate group sizes. More recently, we have also been using the NC3Rs' Experimental Design Assistant to confirm our experimental designs - this tool provides helpful experimental design suggestions (particularly regarding key experimental factors such as randomisation, group blocking and blinding) and a rigorous "sense check" that our design and analysis strategy is the most refined available.

In a limited number of studies, we are guided by appropriate health authorities (e.g the UK Medicines & Healthcare Products Regulatory Agency or the US Food & Drug Administration) to apply certain design principles. Such guidance is often more conservative than, for example, current NC3Rs expectations and tends to modestly increase numbers of animals used (e.g. for some studies minimum group sizes of 10 are stipulated).

What other measures apart from good experimental design will you use to minimise numbers?

Wherever possible we aim to be efficient in our studies - maximising the amount of scientific data we are able to generate from each in vivo study. For example when culling animals at the end of a study it is often possible to retain blood and tissues that can act as suitable control samples for unrelated studies. Before embarking on complex multi-group studies we routinely use historical data and pilot studies to estimate group sizes.

When developing novel traditional chemical drugs, the use of computer modelling is proving to be a helpful tool to replace some animal studies; particularly replacing studies aimed at understanding how long the drug will last. Regrettably, due to the complex biological nature of the drugs we are developing (typically our drugs are a modified virus) and the extended duration of action (months/years rather than hours/days for chemical drugs) computer modelling is not currently a helpful tool to replace in vivo studies.

The PPL includes protocols to support breeding of specific disease-related animal models. Wherever possible, we use refined models that allow highly efficient homozygous breeding strategies to maximise the numbers of animals that may be used on other protocols within the PPL and minimise the breeding of animals that are unused.

A retrospective assessment of reduction will be due by 07 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

We will use mice for all of the studies in this PPL. We have considerable experience in the development of gene therapy formulations for use in human clinical trials and have found our mice studies to be highly informative.

During the planned studies we often deliver our gene therapy formulations to the lungs, muscle and blood stream of these mice. The delivery methods we use - sniffing droplets of liquid into the lungs, breathing in aerosols or injections are often used both in animal research and as ways of delivering drugs to humans. These delivery methods cause no more than brief discomfort.

The gene therapy formulations we deliver can cause mild to moderate flu-like symptoms. These symptoms last at most for a few days and the animals spontaneously recover from them.

For the majority of studies under this PPL we will use normal laboratory mice, However, we will also use several animal models of diseases which cause significant harm to humans. The diseases chosen are genetic diseases that are typically passed on from unsuspecting parents to their children and which either cause an early death or significant health problems in later life.

One of these diseases is Cystic Fibrosis (CF) which causes an early death due to repeated lung infections. We will use an animal model of CF to test if our gene therapy formulations can correct the lung problems caused by CF. In REDACTEDs we have performed related studies - these have led to the development of a gene therapy formulation which has been shown in CF patients to halt lung decline. We anticipate that studies under this PPL will identify an even more potent gene therapy approach which will not only stop the disease getting any worse but will actually improve patient well- being.

Another one of the disease areas is called: Childhood Interstitial Lung Disease (chILD); a collection of disease syndromes that affects the alveoli (air sacs) of the lungs. We may use three animal models of chILD with deficiencies termed SP-B, SP-C or ABCA3. There is variation in the severity of chILD depending on which of these deficiencies a newborn human has inherited from its parents. The animal models of these syndromes mimic this well as the SP-C and ABCA3 models (like their human counterparts) have mild lung problems. We anticipate that studies under this PPL will identify a gene therapy approach which will halt and even improve the problems we see in SP-C/ABCA3 animals models and ultimately patients.

In contrast, the SP-B model is severely affected and dies due to respiratory failure - this is exactly what happens to newborn humans suffering from SP-B disease. Stopping this very

severe disease is the aim of our SP-B studies. If we can stop the harm seen in the mouse model, we believe there is a good chance we can stop children who suffer from SP-B disease from dying. Currently there is no humane endpoint that the research community has been able to develop for this animal model - however, we will attempt to develop such endpoint during this PPL so that we and other scientists can cause less distress to such animals when trying to develop treatments for this tragic disease.

Another disease area we are researching is called A1AT-deficiency. Interestingly, A1AT is naturally made in the liver but is exported and makes it way to the lung where it helps to keep the lungs healthy after they suffer from infections. Humans who inherit a defective A1AT gene from their parents have both liver and lung problems. We will use gene therapy formulations than can make extra A1AT in either the lung, liver or muscle to attempt to stop A1AT disease problems. To test this approach we will use A1AT models that have mild lung and liver problems - which we anticipate we can halt with an effective gene therapy.

The final genetic disease area is Wilson's Disease (WD) which causes an imbalance of copper levels in the. Animal models of WD have mild liver and cognition problems which are also seen in the human condition. We will use gene therapy formulations than can alter copper transport by introducing genes into either the liver or muscle, in an attempt to stop WD problems.

Why are we developing treatments for all of these diseases at once? Historically in our laboratory we have developed a series of gene therapy formulations which have been really effective at introducing genes into the lungs of patients with CF. We anticipate that these formulations might also be effective in other lung disorders - hence we are now looking to develop better treatments not just for CF but also other lung diseases such as chILD, and A1AT-deficiency. We have also discovered in the laboratory that our gene therapy formulations are good at delivering genes to liver and muscle cells. Hence we intend to try these delivery routes for some of the proposed diseases (especially A1AT-disease and WD).

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

We have considerable experience developing gene therapy formulations for the treatment of CF and have translated findings from studies performed on REDACTEDs to multiple clinical trials which have involved hundreds of patients.

During development of these treatments we are continually challenged by both our scientific peers and the government agencies that control the development of new medicines to show that our gene therapy formulations work effectively. This is a pre-requisite for evaluating them in humans. We do use less- sentient and non-sentient model systems in the first stages of our drug development programme, but to- date, we have been unable to identify less sentient model systems than the mice described above that satisfy these demands.

One reason behind this inability to use less sentient systems is that the drugs we are developing are complex biological molecules - for example they frequently contain portions of viruses. Understanding how these complex biological molecules interact with a system (such as an animal) that has an intact immune system. Furthermore, it is highly desirable to understand the duration of any effects caused by our gene therapy formulations; such

studies may take many months - such long-term studies cannot readily be achieved in anaesthetised animals.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

The aspect of this PPL that is associated with most harm is the experimental use of the SP-B mouse model which if left untreated, dies due to respiratory failure - this is exactly what happens to newborn humans suffering from SP-B disease. Stopping this very severe disease is the aim of our SP-B studies. Currently there is no humane endpoint that the research community has been able to develop for this animal model. As well as attempting to develop new treatments for SP-B disease, we are also committed to develop a suitable, scientifically sound, humane endpoint for this model. From the scientific literature, we have learnt that this model shows increasing frequency of breathing and other signs of increased respiratory effort -understanding how these symptoms relate to the severity experience by such animals will be our initial focus. Thus while we intend to use only small numbers of such animals, whenever they are used, we will incorporate increased observations to enumerate these symptoms and attempt to build a predictive score that we could use to terminate a study with knowledge that that any given animal was now unable to recover - in that way we hope to minimise suffering whilst retaining sound statistical evidence supporting any correction of the disease phenotype.

Importantly, the vast majority of studies under this PPL are anticipated to induce greatly decreased animal welfare issues. Nevertheless, where we identify (e.g. using distress scoring sheets for each animals and protocol) areas where refinements (e.g. in pain management) we have included sufficient procedural flexibility to immediately introduce steps that address the issue.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Improving quality of science through better animal welfare: the NC3Rs strategy
https://doi.org/10.1038/laban.1217

Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches

European Medicines Agency - EMA/CHMP/CVMP/JEG-3Rs/450091/2012

How will you ensure you continue to use the most refined methods during the lifetime of this project?

The team of scientists working on this PPL attend regular meetings organised by our local vet services team to make sure we are aware of general 3Rs improvements.

Under REDACTEDs we have been praised by local ethical review panels for both our responsiveness to 3Rs initiatives and for project-specific 3Rs adaptations we have implemented ourselves - for example our recent focus on ALI, EVLP and PCLS studies along with our uptake of in vivo imaging has resulted in a large drop in animal usage. Going forwards we will continue to strive for self-identified improvements in this area and remain vigilant to identify pertinent advances made by others.

Explain the choice of species and the related life stages

Mice have been selected for the proposed studies as they provide a model replete with the full range mammalian cell biology processes. Of particular importance is that they have an intact immune system; this is critical for understanding how recipients of the gene therapy drugs we are developing (animals in this PPL, people in future clinical trials) will respond.

For many studies, normal laboratory mice will be used. However, we also anticipate using a range of genetically altered mouse models. These will be bred under two protocols in this PPL. Each of the models selected present a disease related physiological defect. Clinical development of a novel gene therapy treatment is greatly facilitated by demonstrating that such defects can be corrected by the proposed treatment approach.

Mice harbouring knockouts/mutations of the murine Cftr gene with genetic correction of the CF intestinal defect will be used for studies relating to the development of therapeutic agents to treat Cystic Fibrosis (CF).

Mice harbouring knockouts/mutations of the murine Sftpb, Sftpc and/or Abca3 genes will be used for studies relating to the development of therapeutic agents to treat Childhood Interstitial Lung Disease (chILD).

Mice harbouring knockouts/mutations of the murine Serpina1 gene will be used for studies relating to the development of therapeutic agents to treat A1AT-deficiency.

Mice harbouring knockouts/mutations of the murine Atp7b gene will be used for studies relating to the development of therapeutic agents to treat Wilson's disease (WD).

Mice harbouring standard laboratory reporter genes and/or additional copies of the human versions of the disease genes listed above will also be used. In particular under this PPL they will be used to assess the ability of gene editing approaches to precisely (or otherwise) correct specific undesirable human sequences.

For breeding, all life stages of animals will be used. This is necessary for effective maintenance and use of the genetically altered mouse models.

Adult mice will be used for the vast majority of studies where assessment of gene therapy formulations is anticipated. In a very small minority of studies gene therapy formulations will be delivered to 1-2 days old mice and/or juvenile mice; such studies are of particular relevance to the development of disorders such as chILD and WD where the human disease develops shortly after birth and the target organ (lung and liver respectively) undergo considerable growth in the neonatal/juvenile period .

A retrospective assessment of refinement will be due by 07 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

19. Glutamate Receptors and Epilepsy

Project duration

5 years 0 months

Project purpose

None selected

Key words

Epilepsy, Glutamate Receptors

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Currently, the processes by which epilepsy is established, and why, in some cases, it becomes resistant to drug treatment, are poorly understood. The aim of this project is to advance understanding of underlying mechanisms within the neuronal circuits of the brain that are responsible for generating and maintaining epileptic activity. The work aims to identify how disruption to neurotransmission alters normal synaptic communication between neurons within local circuits of the brain and triggers epileptic activity. In doing so, the project's long-term aim is to identify treatment strategies that could be used to restore normal brain function in patients suffering from drug resistant epilepsy

Retrospective assessment

Published: 6 March 2024

Is there a plan for this work to continue under another licence?

No

Did the project achieve its aims and if not, why not?

No work was conducted under this licence.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The project will advance fundamental understanding of how these neuronal pathways and neurotransmitter receptors are involved in normal brain function, and how these become altered in epilepsy. The information will be of value to scientists working in the field of neuroscience, and specialist medical practitioners dealing with patients suffering from neurological conditions. In the longer term the information generated in these studies will be of value to the pharmaceutical industry in their quest to develop new medicines for the treatment of epilepsy and other neurological conditions.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

The project will run for 5 years. Experiments will be conducted on rats and mice, of which the study is expected to use up to 3850.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

The studies will require the induction of epileptic activity in healthy rats and mice. Consequently, whilst some animals will undergo surgical procedures, performed under general anaesthesia, these are not expected to have any long-term detrimental effect on their wellbeing. In order to study the changes in brain function that occur during epileptic activity, suitable models of epilepsy will be used. In all cases we will apply the least severe model and endpoint suitable for the study, such that no animal will be allowed to progress beyond unnecessary stages of the disease. In the event that any animal develops signs of distress or ill health, it will be killed promptly to prevent any undue suffering. At the end of the experiment all animals will be killed as it would not be legally possible to rehome them under the terms of the Animal Scientific Procedure Act.

Retrospective assessment

Published: 6 March 2024

What harms were caused to the animals, how severe were those harms and how many animals were affected?

No work was conducted under this licence.

Replacement

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

It is not currently possible to study how the brain functions in cell culture or using a computer-based models. Consequently. It is essential to use animals for this study. Equally, it is not possible to undertake this work in a non-protected species as these do not have comparable brains to humans.

Retrospective assessment

Published: 6 March 2024

What, if any, non-animal alternatives were used or explored after the project started, how effective were they and are there any lessons worth sharing with others?

No work was conducted under this licence.

Reduction

Explain how you will assure the use of minimum numbers of animals.

The experimental group size has been estimated using statistical “Power Analysis calculation” based on date obtained from similar studies conducted previously by my research group.

Animals numbers will be minimised by:

Defining effective drug concentration using tissue culture or organ preparations prior to the animal experiments.

Using pairs of independent stimulating and recording electrodes. We will use one pathway as control and the second as the experimental pathway, therefore reducing animals used by approximately 50%.

Making use of historical data to minimise the number of control animals used.

Interleaving experimental groups to enable the control animals to serve more than one set of experiments.

Retrospective assessment

Published: 6 March 2024

How did you minimise the number of animals used on your project and is there anything others can learn from your experience?

No work was conducted under this licence.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

In these studies, we will use rat or mouse which will be submitted to one model of epilepsy. These species have been used extensively in neuroscience because their brains share many similarities to that of humans at both an anatomical and molecular level. The epilepsy models used in these studies are all well characterised and have been shown to reproduce specific aspects of the neurological conditions of interest. In all cases the progression of the disease will be carefully monitored, and animals will not be permitted to progress beyond the early stages of the condition.

All surgical preparations will be performed under general anaesthesia using full aseptic precautions. For recordings taken from conscious animals the connection cables allow the animal to use a full range of normal behaviours and does not restrict movement. Animals will be habituated to the recording room/arena and behavioural apparatus. Experience shows that animals rapidly learn the routine of daily recording sessions, and within 2-3 days will become relaxed enough to perform the experiments

Retrospective assessment

Published: 6 March 2024

With the knowledge you have now, could the choice of animals or models used have been improved at all? How did you minimise harm to animals during the project?

No work was conducted under this licence.

20. Imaging of Cardiovascular Diseases

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	Pregnant, adult
Rabbits	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The aim is to develop novel molecular contrast agents and imaging technologies for application in patients, and obtain the requisite information to determine whether they can be applied in humans. This overall aim falls into two key elements:

To determine, in vivo and in explanted tissues, the biodistribution, pharmacokinetics, targeting efficacy and mechanisms of novel contrast agents/therapeutic agents which have been developed with a view to application in humans with cardiovascular disease, for radionuclide, magnetic resonance, optical and X-ray imaging. To inform a decision on whether biodistribution studies or trials in humans, are justified.

To test in vivo and in explanted tissues, and provide proof of principle for, novel imaging techniques and instrumentation developed with a view to application in humans or animals (e.g. MR dynamic contrast, dynamic time dependent imaging, combined imaging modalities, or methods of correction for motion).

A retrospective assessment of these aims will be due by 23 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Heart and circulatory diseases remain the most common cause of death in the UK and are responsible for approximately 240,000 deaths per year. Coronary heart disease is the most common single cause of death affecting about 117,000 people per year. Heart attacks frequently occur without preceding clinical symptoms but suddenly can lead to life threatening complications and approximately half of patients affected by a heart attack die immediately often before reaching hospital. The purpose of this project is to develop experimental animal models of cardiovascular disease to be used in proof-of-concept and validation studies of novel imaging methods, contrast agents and devices for earlier and more accurate diagnosis of heart disease, to better guide interventional treatment and to monitor how patients respond to therapy. If heart disease can be detected earlier with more understanding of the underlying pathology, this may lead to better medical and interventional treatment of patients and eventually to a reduction in the disabling effects of heart disease and improved survival.

What outputs do you think you will see at the end of this project?

The project outlined will allow us, with the help of disease models, to develop novel imaging techniques and devices for image guided intervention that will help to better diagnose and quantify the severity of atherosclerosis (plaque build up in arteries) and myocardial ischaemia (lack of oxygen to heart muscle due to blockage of blood vessels) and to improve current interventional procedures for the treatment of cardiac arrhythmias, congenital heart disease and heart failure. It thereby may allow us to more efficiently treat patients with coronary heart disease and help to assess how patients respond to treatment. This knowledge will help us in identifying patients that are at high risk of a heart attack and provide the appropriate treatment to patients based on more quantitative and objective measures. Ultimately this study aims to minimise the number of heart attacks by early detection and interventional therapies and improve the efficacy of interventional treatments to cure atrial fibrillation (irregular heart beat) and heart failure.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The expected immediate benefits of this research are the ability to make an informed decision whether to test the new contrast agent in humans, or to abandon the agent, or return to the in vitro or chemical laboratory for further modification.

Better contrast agents and chemistry will improve the quality of imaging (e.g. by improving structural homogeneity, improving affinity, reducing blood clearance and excretion times thus improving target-to- background ratios, giving better detectability of smaller disease). Making radiochemistry of labelling simpler and more robust will lead to shorter production times and hence better efficiency (less technician time, less radionuclide waste by decay), and in turn to wider availability to more hospitals without costly cyclotron and radiochemistry equipment and radiochemical expertise.

Thus with more widely available contrast agents, more patients will benefit from the technology available. We expect several new imaging agents to be translated to human trials as a result of this programme of research. In addition, the benefit of validating novel imaging techniques and devices for image guided intervention will help to better diagnose and quantify the severity of myocardial ischaemia and to improve current interventional procedures for the treatment of cardiac arrhythmias, congenital heart disease and heart failure.

Whether directly by the development of new imaging technologies, or indirectly by use of imaging as a tool in basic biomedical research, better quality and wider availability and applications of imaging technologies will lead to better clinical decision making and better quality of life for patients, reduced drug development costs, and reduced costs for health services. Once these diseases have been identified in a patient, imaging also has the potential to non-invasively evaluate therapeutic efficacy, providing rapid feedback on therapeutic or interventional effectiveness. The beneficiaries will be patients, health services and pharmaceutical companies.

How will you maximise the outputs of your work?

We will closely collaborate with industrial partners to ensure that the developed imaging probes can be tested in phase II and III studies for safety and efficacy and ultimately be commercialised to ensure widespread clinical use of the developed probes for patient benefit. Similarly, we will closely collaborate with industrial partners to develop prototype software and hardware that can be subsequently shared with other academic centres for wide spread clinical testing. We will also disseminate the results of this work at conferences and workshops and publish in peer reviewed national and internal scientific journals. In addition, we will organise workshops at REDACTED where we will share our results and provide hands on training for REDACTED and external academics interested in this work. Other means of dissemination will include talks at the Pint of Science initiative or active participation in the summer festival of the Royal Society.

Species and numbers of animals expected to be used

- Mice: 14000
- Rats: 1500
- Rabbits: 250

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Typically, an animal in this Project License will be:

Induced to express a certain cardiovascular disease including atherosclerosis, aortic aneurysms, myocardial infarction, heart failure, cardiotoxicity and vascular remodeling by using special diets, surgical or pharmacological interventions.

Imaged in vivo by MRI, PET, SPECT, OCT, IVUS under anaesthesia either inhalable or injectable to detect cardiovascular diseases with or without administration of contrast agents.

Treated with therapeutics for modulation of cardiovascular diseases.

Humanely culled.

The duration of the experiment will depend on the Aim of the study and the individual Protocol used. However, all protocols have been refined to that the minimum experimental duration to achieve the scientific objectives.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

The expected impact on the animals includes the induction of cardiovascular diseases including atherosclerosis, aortic aneurysms, myocardial infarction, cardiotoxicity, heart failure and vascular remodeling in a reproducible and controlled manner.

Typical adverse effects for the animals include:

1. Weight: Weight loss will be assessed in comparison to age-matched control animals wherever possible. To reduce animal use, control animal weights will be recorded and pooled into growth curve data that are specific to the strain and the animal unit.

Weight loss of 10% or more in the absence of other clinical signs (see below) will result in increased monitoring and supportive measures such as wet mash diet.

Weight loss of 15% in the presence of other clinical signs (see below) will result in humane killing.

Weight loss of 15 - 20 % in the absence of any other clinical signs will result in humane killing.

Posture: Continuous hunched posture or repeated phases of intermittent hunched posture over a period of 24h or prostration will result in humane killing.

Food/fluid intake: Food and water consumption of 50% as compared to normal average intake over a measurement period of 48h will result in humane killing.

Diarrhoea: Intermittent diarrhoea is defined as diarrhoea that lasts less than 12h. Intermittent diarrhoea for a period of 12h will result in intervention in the form of increased

monitoring frequency and rehydration. If the diarrhoea does not improve within the following 72h despite intervention, the animal will be killed humanely.

Coat: Animals with a staring coat with marked piloerection will be humanely killed.

Behaviour: An animal showing subdued behaviour, even when provoked, and little peer interaction will be humanely killed.

Body temperature: persistent hypothermia for 48h.

Abdomen shape: Animals showing abnormal visible distension of the body TOGETHER with weight increase AND abnormal breathing will be humanely killed.

Cardio-respiratory distress (rapid breathing, tachycardia, wheezing) would result in humane culling.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The expected severities and proportion of animals experiencing each severity includes:

Mild 60%

Moderate 38%

Severe 2%

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 23 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We require the use of animals because:

Data generated from this body of work may be used to inform whether to go forward to human clinical applications. Regulatory agencies require animal data to demonstrate safety and efficacy before molecular imaging agents or therapeutics (that can be validated through imaging) can enter human trials.

To validate the mode of action new/improved molecular imaging agents, experiments are required that cannot be conducted in humans for ethical and scientific reasons (e.g. contrast agent distribution requires intact physiological barriers and excretion mechanisms).

Bio-distribution in whole organisms (i.e. tracking the injected agents route/ accumulation and excretion through the body), with intact biological barriers and excretion mechanisms, is key to clinical use.

Most aspects of cardiovascular pathologies can only be studied in live animals (e.g. atherosclerotic plaque progression, aortic aneurysm expansion, myocardial infarction and cardiac function) because there are complex interactions between different body systems, which cannot be replicated in anything other than an intact animal.

What was your strategy for searching for non-animal alternatives?

Most of the contrast agents to be studied have not been used in man before and require animal data before approval for human studies. In many cases, even if the contrast mechanisms are well- established, it is unethical to use the tools (e.g., inhibitors) required to validate the corresponding targeting mechanisms and to understand the biological mechanisms in humans. However, in some limited cases, absolute replacement using humans is a possibility, e.g. when contrast agents that are already used in humans are evaluated for clinical utility and uptake mechanisms. If this is feasible and allowed by the regulatory agencies we will do so.

Although, non-animal alternatives cannot replace the complexities of the interactions of these probes in whole body systems or with realistic models of cardiovascular disease, prior to all in vivo work, human and animal cell- and tissue-based methods will be used as relative replacements to answer as many research questions as possible and build solid hypothesis to be subsequently tested in vivo. For example, this includes ex vivo experiments designed to (objective 1) determine target-binding efficiency, agent toxicity to cells, agent stability in cells/tissues/serum; (objective 2) phantom experiments to demonstrate function and capability of the new instrument. Any new/improved molecular imaging agents or instruments, which are found by ex vivo experiments to be unlikely to succeed in animal or later human trials will be eliminated at this stage

Why were they not suitable?

Alternatives apart from those listed above, are not suitable because non-animal alternatives cannot replace the complexities of the interactions of these probes in whole body systems or with realistic models of cardiovascular disease.

A retrospective assessment of replacement will be due by 23 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated the number of animals required based on published information about the proposed animal models regarding the reproducibility and prevalence based on our own pilot data whenever possible.

A typical study might be planned as follows:

For testing a new molecular imaging agent in mice (Objective 1), we estimate animal numbers using a power calculation (two-tailed t-test, $\alpha=0.05$, power=0.9, typical measurements of 7.8±3.5(signal) and of 2±1(noise) yielding $\delta=5.5$ with $\sigma=3.5$) yielding N=9 animals/group. To account for complications with anaesthesia, specific diets, interventions or treatments, we would plan to investigate 10 animals/group. If target-to-background (signal-to-noise), or difference between experimental and control groups, is expected to be large (>1 order of magnitude change, e.g.in the case of using effective contrast agent blocking substances), or found to be so by pilot experiments, statistical significance may be achieved with fewer animals/group. However, we will not use less than 3 animals per group to comply with generally accepted scientific reproducibility criteria in the field.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Pilot experiments will be performed where necessary on small cohorts, to provide statistical data allowing animal number estimations for definitive larger experiments. Where possible, multimodal imaging will be used to multiplex and study two or more agents/mechanisms simultaneously in the same animal. Multimodal cross-validation will yield superior data because each animal will be the control for itself – data will be intrinsically paired. Sometimes, it will be possible to study >1 vascular pathology per animal (e.g., vascular remodeling and plaque progression in 2 different vascular segments), with the same advantages.

What other measures apart from good experimental design will you use to minimise numbers?

Imaging to determine tracer distribution rather than conventional ex vivo organ counting is a major contributor to reduction. It allows repeated time-dependent measurements on the same animal as animals are only killed at the last time-point; for example, if a study involves six time-points, the animal numbers are reduced to one sixth. Since each animal serves as its own control, the data are statistically also more robust, which in turn leads to further reduction as smaller cohort sizes are required (because inter-animal variability no longer needs to be considered at the experimental design stage). Moreover, not only contrast agent distribution in vivo, but potential time-dependent and unexpected re-

distribution can be detected through serial imaging. All these attributes contribute to a greatly improved benefit: cost ratio (benefit=data quality/quantity, cost=animal numbers/procedures).

A retrospective assessment of reduction will be due by 23 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Species: Mice, rats and rabbits are the species of least neurophysiological sensitivity that provide the capability to support the development of various cardiovascular diseases (e.g., atherosclerosis, aortic aneurysm, myocardial infarction) and the minimum size compatible with the scale of resolution/movement associated with the imaging technique. Resolution of whole-body imaging techniques is of the order of 0.1-1mm.

Animal models: We will use cardiovascular disease models established through (i) use of genetically altered or wild-type animals that develop atherosclerotic in combination with high-fat diet, (ii) genetically altered or wild-type animals that develop aortic aneurysm in combination with chemical or pharmacological compounds, (iii) genetically altered or wild-type animals that develop myocardial infarction through surgical occlusion of the coronary artery, permanent or transient, (iv) wild-type rabbits that form atherosclerosis by a combination of surgical injury of the aortic endothelium and high cholesterol feeding and plaque rupture and thrombosis by pharmacological triggering, (v) genetically altered or wild-type animals that develop vascular remodeling following mechanical injury of the vessel wall, (vi) genetically altered or wild-type animals that develop cardiac diseases using chemotherapeutics, (vii) genetically altered or wild-type animals that develop vascular calcification with special diets and compounds. Only the models and progression stage that are key to a required outcome of each project goal will be used.

Methods: The use of cardiovascular models is necessary to pre-clinically validate new or improved cardiovascular-specific contrast agents. New and improved contrast agents will be validated in this project and hence, must be used in conjunction with the appropriate models. To thoroughly validate new contrast agents for later translation into human trials, the use of a variety of cardiovascular models is required. Their use is also required to perform preclinical studies in vascular biology including disease progression, vascular heterogeneity and evolution, and treatment response. To study vascular biology and exploit the power of existing and emerging imaging methodology, new and improved contrast agents are required to obtain the necessary imaging data. Differing cardiovascular

models such as those of disease progression in mice and plaque rupture in rabbits are necessary to study progression and clinical end-points; in this context repeated imaging with specific molecular imaging contrast agents is a very powerful method to quantify atherosclerosis evolution and molecular changes that lead up to a clinical event over time. Various animal preparation approaches to define and control the development of cardiovascular diseases are essential for these studies; this includes modification of the lipoprotein system (either by using genetically altered strains or by special diets) and/ or surgical interventions that cause injury to the vessel wall or the heart that trigger disease. Specific insight into molecular processes can be obtained either via genetic approaches (genetically altered animals) or via pharmacologic approaches (e.g. inhibitor molecules). To discover what treatments might reduce cardiovascular diseases the use of animal models together with therapeutic approaches is required, whereby repeated imaging serves as a very powerful method to inform on time-dependent changes in the very same animal; administration of therapeutics is mandatory in this setting. Moreover, cardiovascular models must be employed to pre-clinically validate new imaging instrumentation that is specifically developed for use in relevant applications. Common to all imaging methodology is the requirement of the animals remaining motionless during imaging, which renders general anaesthesia essential. General anaesthesia is also necessary for surgical procedures (e.g. myocardial infarction, vessel wall injury).

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Less sentient animals cannot be used as they do not have a fully developed cardiovascular system to replicate the complexity of the diseases of interest.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Minimising suffering: Animal cardiovascular disease models are set up to develop cardiovascular pathologies and complications. As vascular and cardiac pathologies and cardiac disease progression and regression as well as the development of new contrast agents specifically aimed at the detection of molecular processes are goals of this project, we require to progress disease to late stages of development stages; also, treatment responses requires first that cardiovascular diseases are in place, which need to be treated (as compared to untreated control cohorts) to mimic realistic situations as normally relevant to human treatment. Animals will be monitored daily at early stages post-surgical interventions and at least three times weekly once early clinical signs have developed.

It is a part of PPL conditions and our ethical understanding that we let animals only develop cardiovascular diseases to a level of burden required to answer the specific research questions of each individual experiment, for example: (i) if initial in vivo experiments are performed to investigate the uptake of a new contrast agents in early atheroma, the answers can be obtained by the presence of relatively small plaques without the need of advance disease or plaque rupture; (ii) the use of repeated imaging in disease progression and treatment experiments where we then can quantify vascular and cardiac burden by imaging and obtain statistically better data (repeated imaging reduces suffering by reducing the required animal cohort sizes). In fact, imaging will help us in many cases to better assess the overall disease burden in our animals. Once an individual experimental goal is reached the animals will be culled regardless of whether the endpoint has been reached.

Inhalation anaesthesia will be used wherever possible to minimise transient pain and distress, e.g. during imaging. In addition, full recovery between periods of anaesthesia, rehydration during long imaging sessions, respiration/cardiac function monitoring, body temperature monitoring/maintenance will be conducive to animal wellbeing. If food withdrawal is required for metabolic studies, we will use further spaced out repeated imaging to allow the animals to take in food ad libitum for a minimum of 28h before the next session of food withdrawal.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We will follow established published guidelines to ensure experiments are conducted in the most refined way. These includes:

The Responsibility in the use of animals in bioscience research produced by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).

The Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986.

Accepted limits of volumes and frequencies when administrating compounds and anaesthesia (Appendix 1a in Action Plan section).

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We will stay informed by updates from the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) website and seminars on the 3Rs organised within and outside of our institution.

Additionally, we have direct support and contact with an NC3R's regional Programme Manager who supports the application of the 3Rs at REDACTED and is a member of the Policy and Outreach Group. This includes providing expert advice and coordinating the sharing of best practice.

Explain the choice of species and the related life stages

Adult murine, rat and rabbit models of cardiovascular diseases are used in this Project License because they reproduce many of the traditional cardiovascular risk factors associated with the development of atherosclerosis, aortic aneurysms, myocardial infarction and heart failure, vascular remodeling as seen in the corresponding human conditions.

A retrospective assessment of refinement will be due by 23 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

21. Infection and Immunology

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	adult
Rats	adult
Guinea pigs	adult
Ferrets	adult
Rabbits	adult
Cotton rat	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The aim of this project is to test and develop anti-infective drugs and vaccines as a service for customers. We aim to assess their therapeutic potential in animal models. These tests are carried out to satisfy regulatory guidelines to ensure any drugs and vaccines are safe for use in humans and animals.

The aim is to identify, develop and characterise new treatments with potentially better efficacy, and fewer side effects, than existing medicines and vaccines currently available to doctors and vets.

A retrospective assessment of these aims will be due by 30 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Vaccines and anti-infective agents play an important role in the routine health and wellbeing of both the human and animal populations. Routine vaccinations in humans and animals have largely prevented the spread of significant diseases and their associated illness and death (e.g. mumps, measles, polio, diphtheria).

There is a shortage of (in particular) effective antibiotics currently available to doctors, due to overprescribing of drugs for viral and trivial infections (for example) leading to bacterial resistance. This means that bacteria have found ways of dealing with current drugs which means that drugs don't work as efficiently to kill (bacteria) like they used to. In a short space of time, at the current rate, there will be a shortage of effective antibiotics for a wide range of routine infections, which because of this, will mean that previously minor infections could become life threatening. This project would aim to aid in the development of new agents, possibly of new chemical classes, that maybe less prone to these resistance mechanisms in bugs.

Similarly, due to the increasing use of drugs that suppress the immune system in patients with cancer/HIV and other indications, fungal infections in the bloodstream and lungs for example are increasing. Standard therapies at the moment are often toxic in patients (e.g. one standard antifungal treatment causes renal damage) who are already ill. There is an unmet need for newer, safer, Anti- fungal drugs with better side effect profiles in such vulnerable, sick patients.

Vaccination protects the public from serious illness and complications of vaccine-preventable diseases which can include paralysis of limbs, hearing loss, convulsions, brain damage, and death. This project will aim to develop vaccines for a wide range of conditions that are safer and more effective than existing vaccines currently in human and animal use. Progress has already been made with therapeutic cancer vaccines (e.g. vaccinating young girls against a virus that can cause cervical cancer) and future potential targets include addiction, diabetes, high blood pressure and Alzheimer's disease, as well as for other targets such as allergies.

Where vaccine immunogenicity is tested, as they are biologics there could be a request to assess the biological activity of batches-this would only usually be for vaccines that were being used in humans, and for safety and quality purposes.

What outputs do you think you will see at the end of this project?

The short term outputs of this project will be the provision of high quality data , which will be used to make decisions as to whether to progress anti-infective agents and vaccines further, possibly into clinical trials. It will also provide data which may prevent further testing in animals, and hence an overall reduction in the numbers of animals used.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

In the short term, sponsors would benefit from the data produced, as it will enable them to make critical stop go decisions regarding their drugs or vaccines, and/or use the data produced to add to regulatory submissions to enable their drugs or vaccines to progress into clinical trials and possibly gain market authorisation.

The public or animals may benefit from this work, as it may lead to new products reaching both the animal and human market, with better and longer lasting activity, better treatment outcomes, and with less side effects.

Some of this work will be carried out under the Good Manufacturing Practice regulations, which will ensure that any vaccines or peptides tested are high quality, safe and fit for use in humans or animals.

How will you maximise the outputs of your work?

The work will be shared with customers who will use it to determine their future drug and vaccine development strategy, or for submission in documents required by regulatory authorities. This organisation has no control over what happens to the data after we have shared it. Previously however, we have collaborated with customers and shared data we have produced in the form of Scientific publications.

Some customers do use the data we provide for stand alone publications, although this is not always communicated to us.

Species and numbers of animals expected to be used

- Mice: 17000
- Rats: 8500
- Guinea pigs: 2250
- Ferrets: 3000
- Other rodents: No answer provided
- Rabbits: 1200
- 2500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

In studies involving the immune system, an animal will typically be vaccinated with a material (maybe in a muscle, intravenously, or under the skin), which may be boosted with one or more further injections (typically two or three but maybe more) at a later stage. Either before or after the first dose, blood samples will often be taken (so we can measure and track the immune response e.g. production of antibodies). After a period of time (Typically 1-3 months) the animal will be killed, and organs like the spleen will be taken to measure a further immune response in a tissue.

In infection studies (typically lasting 48-96h, but up to 14 days or beyond), animals will be infected either systemically (e.g. in the lungs, intravenously) or maybe in the thigh, with a bacteria or virus or fungi (we will know the correct dose to give after we read scientific papers or small pilot experiments we've already carried out). They will then be treated with a drug that we know from test tube work will kill the organism we are inoculating. The animals will then be carefully observed after we've infected them. We will measure temperature and record clinical signs, and continue dosing with the test drug. Occasionally we may take some blood or other samples too. If the animals get too sick (in most experiments this won't happen because we've already tested the infection at this dose), we will kill them to stop any further suffering. We will continue to monitor them depending on how sick they are right up until dosing ends and the experimental time is up. At that point we will humanely kill the animals and prepare organs e.g. the lung or the kidney to count the number of organisms present, and see if the treatments were effective.

Injections and blood samples that are carried out in animals are a very similar experience to what a patient would have when having the same done by a doctor or a nurse in a GP's surgery or hospital. To do this we often have to restrain animals for a short period of time, in a purpose built device, or by hand, so that we can collect samples or inject animals without overly harming the animals (like if they start to struggle).

Whilst this describes what typically happens, on some (very rare) occasions we may use mice that have been genetically modified to better model human disease. We may also remove food and water from animals for short and controlled periods of time to aid with dosing new drugs. For both occurrences, this may happen once or twice in a 5 year period.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Animals in most infection models may have symptoms like being cold, their hair standing on end and closed eyes. They will also lose weight. In animals that are treated with drugs, most of these effects will either be less pronounced or mainly absent, and they will be moving around their cage looking largely normal and eating and drinking and grooming normally. Depending on the way we infect the animals, they can start to show signs of illness from about 12h after we infect up to the end of the study.

Very occasionally we infect animals with a high dose of an organism that may cause it to have severe clinical signs. This is to test how good the drug we are testing is in a model of infection that you may see in a very sick patient in a hospital. We would expect most animals, even ones treated with drugs to show some clinical signs after this, like being cold, lose weight and look ill. In all cases we will humanely kill animals when its clear they will not survive. We observe these animals on these studies very careful, at short intervals, to make sure we can kill them humanely if we need to, or monitor the progression of their illness.

Animals treated with vaccines very rarely show any signs at all. The vaccines are not designed to make them feel unwell. Occasionally we will see some mild signs, sometimes where we inject the vaccine into. But in approximately 99% of animals we see no clinical signs of illness at all. The animals will move around, play and eat, drink and groom as normal.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Overall figures in the last license showed that approximately 95% of animals suffered mild severity, 4% of animals suffered 'moderate' severity and less that 1% of animals suffered 'severe' clinical signs.

During the last license less than 2% of animals infected with a microorganism experienced severe signs, due to the careful chosen doses of infection used, the regular clinical observations, temperature measurements and weighings which will go up if animals are becoming ill due to the infection. Around 10% displayed moderate severity, and 88% mild severity.

On studies that examined the immune system greater than 99% of animals experienced no more than mild severity, caused by the injection of the potential vaccine to cause the immune response. Less than 1% of animals suffered severe clinical signs that resulted in death or humane killing.

We expect numbers to be similar under this project, although this will depend on the specific study types we perform.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 30 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are needed to demonstrate that the drugs or vaccines being tested can work in a mammalian system which is similar to humans. This may mean that we show, for example,

that they can kill bacteria and stop animals developing infections, or show that they can produce an immune response (antibodies) to being treated with a vaccine. If they can't kill bacteria or produce an immune response in animals, then it's highly unlikely they will do so in man.

In some cases, for regulators checking the safety of drugs, or the quality of vaccines, these tests are required to be completed before the drug is dosed in humans, to ensure its safe, or can produce a consistent response when dosed to thousands of people.

What was your strategy for searching for non-animal alternatives?

There are no other non-animal alternatives for the work being undertaken on this project.

Prior to testing in animals, the drugs will have been tested in cells and against microorganisms to check on how toxic they are and how potent they are in killing organisms in test tubes. Drugs that are either toxic at low concentrations or do not kill organisms in test tubes would not be chosen to be tested in animals.

Why were they not suitable?

The intact Immune system is a complex system which is not fully understood and therefore for the work detailed in this project, there is no adequate test tube model to replace the whole animal, that can model all the processes under investigation.

Similarly, infection is a complex disease process involving the immune and inflammatory systems as well as the toxicity of the infective agent on its own. It cannot be adequately modelled in test tubes due to the complex interactions between the infection, the animal, the immune system and the drugs being tested.

A retrospective assessment of replacement will be due by 30 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence). It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals required. For study types that are less well established and for which historical data may not be available, the literature will normally be consulted to help establish the group size.
Alternatively, there may be other data to aid this process. The REDACTED are often consulted to assist in this process particularly where the study type is not routine.

Whenever possible, common control groups will be used in order to minimise the numbers of groups used.

For less established study types, preliminary pilot studies may be conducted whereby smaller numbers of animals may be used to generate data in order to ensure that the experiment operates to expectations and to generate some data which may be used to optimise the study design.

Experience has shown that occasionally, Sponsors have a preference with regard to their design and numbers of animals to be used. Rationale for the design will be requested from the Sponsor and such designs (particularly where they are at variance with EU requirement or studies usually conducted here) will be discussed internally (and the Home Office as appropriate) and forwarded to the Department of Statistics for advice. Such advice will be taken into account when determining the design/numbers to be used in the study with the goal of using the least number of animals to achieve the scientific objective.

What other measures apart from good experimental design will you use to minimise numbers?

For studies where a new drug, or a new infection is being tested in animals for the first time, we would often test that in a small group of animals (usually 3-5) to give us confidence that the dose or inoculum levels we chose are safe, and the organism/ drug affects the system its designed to, without making an animal too ill. These are called pilot studies.

We will also try and get as many outputs as we can from a single animal where possible. So if we need to get a level of infection in the blood and tissue, or if we need to find blood borne markers of infection we will often do that in the same animal, rather than use seperate ones.

A retrospective assessment of reduction will be due by 30 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

For our infection models, our animals are infected either by injection or installation of infective agents into the body (e.g. the lungs). We often start by doing small trials (or pilot studies) in small groups of animals to see the level of infection we see, and make sure its right for what we are trying to find out. Sometimes to mimic what happens in real patients, or to remove the effect of the immune response to infection, we administer drugs to suppress the immune system.

When we infect our animals, they do get sick, as humans do when they have say a chest or other infection, but we watch them very closely, provide them with extra bedding to keep them warm, and sometimes provide special foods and gels to keep them fed and hydrated. When our animals get too sick and we know they won't recover, we kill them humanely to end any suffering. For the vast majority of our infection models, most of our animals suffer only moderate symptoms (weight loss, go cold, look ill and stop grooming a little, but are still active) because we do these pilot studies, and because we watch the progression of their disease after we infect them.

On occasion where we infect with a higher dose of infection, the same observations apply, but we would check the animals more regularly as they get sicker. Some of these animals would die without our intervention, and when it becomes apparent that an animals will not recover based on clinical signs, we would humanely kill them and not allow them to suffer further and die.

Our studies involving checking the immune response to vaccines cause very little distress to animals. They are usually a series of single injections or boosters several days apart. Sometimes there will be blood sampling during the course of the study, but mostly all the samples will be collected under anaesthesia at the end of the study, or after the animal is dead. During this whole procedure more the 95% of the animals would show no clinical signs at all and will be eating and drinking and acting normally.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The vast majority of studies are carried out in mice, which are the least sentient species that have an immune systems similar to man, or give the clinical signs and symptoms of infection seen in patients.

Both immunology and infection models last for at least 24h or very often much longer, and it is impractical to keep animals anaesthetised for such long periods of time, without affecting the answers to the experimental questions we are asking.

We use adult animals to model infection and immune responses as their immune system is mature and responds more predictably in comparison to juvenile animals. Effects in adult animals relate better to what is seen in the clinic as well, where the vast majority of patients are adults.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Rodents and guinea-pigs will be mainly used for the tests conducted under this licence. These species are considered to be of the lowest neurophysiological sensitivity commensurate with achieving the study aims. The ferret may be used for discrete modelling of some infectious disease (e.g. influenza and other viral infections) due to its similarity in clinical signs, and immune and inflammatory responses to man.

Infection models

Generally, for infection models, the use of organ / tissue burden data collection endpoints will be used whenever practicable as due to the nature of the infective titre administered, animals should develop a meaningful infection (relevant to the infectious agent) without displaying severe symptoms of infection. The assessment of organ / tissue burden of a microbiological agent would be made post mortem after a period of infection, though for some infection models the progression of the infection may also be assessed by methods such as; serial blood sampling (e.g. bacterial sepsis model) or swabs (skin infection model).

In rare cases, however, it may be necessary to administer an infective inoculum where a lethal inoculum of a microbiological agent is used to evaluate the effectiveness of a test material. This model would only be performed when the anti infective properties of a test material have already been established e.g. generation of tissue burden data or evidence of immune response in a vaccine.

Measurement of microbiological burden (tissue burden) in an animal will be undertaken as a data end point wherever possible to assess the anti-infective properties of a test material, meaning reduced infective inocula and clinical signs of moderate severity (a pilot study would be performed to optimise the infective inocula) . However in rare circumstances, this may not be appropriate due to the nature of test material (e.g. a vaccine) or specifics of the organism under test (may not be amenable to such studies due to growth characteristics to allow this data endpoint, or its specific interaction with host), the stage of development of the test material, hence the requirement for a model resulting in more severe clinical signs.

For all infection models signs of clinical disease will be carefully assessed by observation and recording of body temperature , with the frequency of observation thereafter being dependent on the clinical condition of the individual animals. Bodyweight will be assessed at least once daily. For models where severe clinical signs are expected, this observation minimum would be reduced, with the frequency of observations again dependent on the individual clinical condition of the animal. In both cases, supportive measures (e.g. extra bedding, more palatable food, food supplements, additional heat) will be employed for animals exhibiting clinical signs.

All animals on infection studies will have signs of clinical disease assessed. If animals are rendered neutropenic prior to infection, they will be checked at least twice daily for the onset of clinical signs.

From the induction of neutropenia the frequency of assessment will depend upon whether animals are exhibiting clinical signs, along with information from published literature. When animals are displaying clinical signs then the frequency of checks will be increased from the normal twice daily to approximately every 6 hours or as required.

Body weights and body temperatures will be measured at least daily after induction of neutropenia.

Supportive husbandry (e.g. extra bedding, food gels, additional heat sources) will also be offered as appropriate.

In some cases, the provision of samples (e.g. blood or nasal lavage in ferrets) which will allow a 'global' assessment of the individual animal condition which would assist in tracking disease progression.

Surgical procedures will be carried out in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) and appropriate

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

For any surgical interventions, then the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) will be followed.

For blood sampling and dosing then the following guidelines/literature will be followed:

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).

A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23 (2001).

Regulatory guidelines

WHO guidelines on nonclinical evaluation of vaccines. Annex 1.

ICH Topic M 3 (R2)
. Non-Clinical Safety Studies for the Conduct of
Human Clinical Trials and Marketing Authorization for Pharmaceuticals.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animals Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

Explain the choice of species and the related life stages

Most of our experiments will be carried out on mice and rats as these are the smallest relevant species that we can use that have an immune system and that respond to infection that is comparable to humans. In some specialist cases we may use other animals (e.g. the guinea pig, hamster, ferret, cotton rat and rabbit) because what we are trying to find out is better done in that particular species rather than to the rat or mouse. For instance you can model human influenza in a ferret, but not in a mouse or a rat, and a ferret produces both the symptoms and immune response to the flu virus as humans do. Human flu viruses are not viable in rodents.

The only other time we would use a species other than a mouse or rat is to continue work that has been previously done in that species. For instance if previous work, and results gained, had been carried out in a guinea pig, it would make no scientific sense to start the next stage of a programme of work in a rat or a mouse.

We will be using adult animals in our studies, as we do not expect to be investigating either immune responses to vaccines or treatments for infections for use in young animals or children.

A retrospective assessment of refinement will be due by 30 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

22. Redacted

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Stroke is a life-threatening condition, occurring when blood flow to the brain is cut off. The treatment is to reintroduce the blood flow (e.g. by surgery or drugs). The re-introduction of the blow flow to the brain involves activated blood vessels, small chemical mediators and immune cells (collectively termed inflammation). Reducing, and ideally eliminating inflammation is critical for recovery from stroke – this process is called ‘resolution’. However, patients who already have other illnesses such as Sickle-Cell- Disease (SCD), a genetic disorder affecting red-blood-cells, are highly susceptible to stroke and show reduced recovery after stroke. Thus, the aim of the project is to induce a stroke in mice with SCD and administer drugs to resolve the inflammation and protect the mice against stroke.

A retrospective assessment of these aims will be due by 20 October 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is important to undertake this work because stroke is one of the leading causes of mortality and the leading cause of disability worldwide. These studies will provide new insight into whether targeting inflammation (i.e. by promoting resolution) in stroke is protective.

What outputs do you think you will see at the end of this project?

Stroke limits the blood supply in the brain, resulting in death and inflammation. This is of particular significance in patients that are highly susceptible to stroke e.g. sickle cell disease (SCD) patients. This PPL builds work outlined in REDACTED. Specifically, during the course of this five-year PPL, REDACTED examine whether there is a disruption of endogenous pro-resolving pathways which may contribute to persistent chronic inflammation and may in part account for the fact that SCD patients are not only more susceptible to stroke but exhibit an exacerbated course of disease as well as clinically poorer outcomes New data obtained will be submitted and published in peer reviewed papers and presented at scientific meetings. Following on from this PPL, the longer term vision REDACTED, once mechanisms have been identified, REDACTED, particularly for patients with co-morbidities such as SCD.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

This project will help us to obtain results that could help us to obtain possible therapeutic targets and drugs for the management of stroke, particularly in highly-susceptible patients e.g. patients with sickle cell disease (SCD).

How will you maximise the outputs of your work?

Throughout this PPL and beyond, collaborations are key to driving science forward. The knowledge obtained through the data collected in the PPL will be disseminated at scientific meetings and will be published in peer reviewed journals. Data will be deposited into repositories such as REDACTED and tissues may be available upon request. New methodologies will be sought throughout the course of this PPL and links with the NC3Rs rodent models of stroke network will be sought (https://www.nc3rs.org.uk/rodent-models-stroke).

Species and numbers of animals expected to be used

- Mice: 3500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Our main objective is to understand the mechanisms behind stroke and why people with certain diseases (e.g. sickle cell disease [SCD]) are more susceptible to stroke and the outcomes of stroke. Thus, in order to study stroke in SCD, we will use mice that are genetically engineered to have SCD and induce stroke in these animals. The stroke model is one which is performed routinely and in many different laboratories including ours. Occlusion (i.e. blocking of a vessel in the brain) of upto 120 minutes will occur using a filament, after which time the filament will be removed so that blood flow can re-introduced (termed reperfusion). Where the reperfusion period is longer than two hours, the animal will be sutured and the animal allowed to recover from anaesthesia (an analgesic agent(s) [for pain relief] will be administered as per NVS recommendations, as clearly stated in the protocol).

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

As is seen in the clinic with stroke patients, mice having had a stroke may experience pain, weight loss and changes in behaviour (due to the damage in the brain from the stroke), which typically occurs over 48h. Rigorous monitoring regimes have been put in place for post-operative care. Aseptic techniques will be performed along with barrier principles and environment management to minimise the risk of infection and analgesics will be administered, as appropriate after surgery. The advice from the NVS/NACWO will be sought as appropriate. All drugs/compounds will be made up in sterile conditions. Environmental enrichment will also be given.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The breeding protocols (protocol 1 and 4) are mild. Protocol 2 is severe due to the fact that like the clinical situation, stroke is associated with mortality. In our stroke model, this is typically around 20-30%. Protocol 3 is moderate due to the fact that several things could happen to an animal i.e. one blood sample taken, anti-inflammatory drug administration, cell depletion, inflammogen administration followed finally by thrombosis. Only 10% of mice on protocol 3 will have this. Typically, a blood sample will be taken, anti-inflammatory drug (and/or control) administration and thrombosis model will be performed. Up to ~750 mice will be used for protocol 1, ~1000 mice in protocol 2, ~1000 in protocol 3 and ~750 mice for protocol 4.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 20 October 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

In order to understand what happens in stroke I will need to perform experiments in-vivo since some of the experiments described are not possible either in humans or in-vitro models. While we have previously used cell culture models to study some aspects of the cell-cell interactions elicited by inflammation, it is not possible to mimic all the complex, multicellular interactions that allow all aspects of a cerebral inflammatory response in stroke. Therefore, I have chosen to use mice since rodents are the lowest species in which stroke can be effectively modelled. Throughout this programme of research I will ensure that I continue to adhere to the principles of reduction, refinement and replacement. I will share tissue samples with other researchers where possible and I will continually reassess possibilities for alternative or complementary experiments.

What was your strategy for searching for non-animal alternatives?

Human cells and in vitro tests.

Why were they not suitable?

In humans and in-vitro it is not possible to perform in depth analysis of all aspects of inflammation that occurs in the brain following stroke. Mice will therefore be used in these studies as rodents are the lowest species in which stroke can be effectively modelled. I will throughout the course of this PPL seek, review and incorporate any alternatives and other R's should they arise.

A retrospective assessment of replacement will be due by 20 October 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers have been estimated based on calculations performed from similarly designed studies (total 3500 mice), showing group sizes need to be in the range of 8-10 mice/group. However, these numbers are based on all experiments working, including the number of mice required for the different drug combinations. It is likely that considerably less animals will be used in this PPL e.g. if the anti- inflammatory drug REDACTED does not work at the concentrations chosen (based on in vitro data and EC50 values), then there will be no necessity in determining the mechanism of action e.g. coadministering the drug with a drug that blocks receptors known to mediate the effects of REDACTED (such as the pan antagonist REDACTED). A realistic number estimate of the number of animals. However, the numbers requested allow for flexibility with respect to the studies proposed i.e. so that we can pursue different avenues depending upon results.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Advice from local statisticians was sought, along with adopting helpful tips and advice form the NC3Rs.

What other measures apart from good experimental design will you use to minimise numbers?

I will share tissue samples with other researchers where possible and I will continually reassess possibilities for alternative or complementary experiments.

In general studies were performed blinded and randomised, with a key system to identify which animal/sample has undergone which treatment. Furthermore, compounds administered will be made by laboratory personnel other than the one performing the experiment. Controls will comprise of a vehicle (often saline) for the respective drug to be tested. Sham animals (i.e. animals that undergo the same procedure as the stroke animals but without the occlusion) will be set up alongside the stroke animals as sham mice are necessary to control against any findings associated with the surgery itself. Sham mice are still required for medium/longer term studies to ensure that the results obtained are true and not due to the surgery itself. There will be less than one sham per surgical occlusion model.

A retrospective assessment of reduction will be due by 20 October 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Clinically 85% of all strokes are ischaemic in origin. We propose to use two different models of ischaemic stroke (i.e. the transient middle-cerebral-artery-occlusion/reperfusion [MCAo/R] model or the bilateral common carotid artery occlusion/reperfusion [BCCAo/R] model). The MCAo/R model replicates an ischaemic stroke within the middle cerebral artery and the BCCAo/R model replicates global ischaemia (e.g. as seen clinically with cardiac arrest with reperfusion). This PPL will be split MCAo/R (~80%) and BCCAo/R model (~20%) because more strokes occur in the MCA region and as such our research focus will be directed more towards this area of research. However, it is important to study both clinically relevant models for possible therapy and stroke management. Different reperfusion times are required because each model involves different being clamped or blocked i.e. BCCAo will be for up to 10 minutes and for MCAo, the MCA will be occluded using a filament for a period of up to 120 minutes

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Humans typically suffer a stroke in their adulthood. As such, to recapitulate the clinical setting in a mouse model, I need to be able to use mice that are at a more mature life stage. In addition, these mice cannot be terminally anaesthetised as I am studying the effects of stroke and drug treatment for the management of stroke in a murine model.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Ophthalmic ointment, appropriate anaesthesia (and possibly reversing agents if applicable) and analgesia will be given as advised by the NVS. Animals will be observed frequently and weighed daily. Animals will also be allowed to recover in specialised housing racks (Tecniplast DBS monitoring rack) in which post-operatively they will recover in a quiet area that will be clean, dry, warm and dimly lit. We will also monitor animals more frequently and outside of working hours, so we can take action quickly. As experiments will be performed on mice that are a model of sickle cell disease (SCD), it is highly likely that this intervention will increase the effects of stroke. All staff looking after these mice will be made aware of the additional risk and animals will be euthanized when indicated to prevent undue pain and suffering.

Compounds administered will be research grade and will have been tested in other in vivo models of inflammation.

In general, studies will be performed blinded and randomised, with a key system to identify which animal/sample has undergone which treatment. Furthermore, compounds administered will be made by laboratory personnel other than the one performing the experiment.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

I will adhere to the Home Office guidance of the Animals (Scientific Procedures) Act 1986 and the NC3Rs guidelines.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

New updates will be sought from the NVS, NCIO and AWERB at the REDACTED and from other agencies e.g. the NC3Rs. Updates that are applicable to the PPL will be implemented where possible.

Explain the choice of species and the related life stages

Fortunately, the biology of inflammation and thrombosis are similar in rodents and humans, thus mice will be used. As we are interested in the reasons why sickle cell patients are highly susceptible to thrombotic events and stroke, we will use mice that have sickle cell disease to study the mechanisms and possible therapeutic targets for reducing inflammation and thrombosis. Typically strokes occur in adulthood (although much younger in sickle cell patients), thus we will use adult mice in this protocol.

A retrospective assessment of refinement will be due by 20 October 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

23. Investigating the genetic control of cancer

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

cancer, genetics

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Deaths related to cancer are the second leading cause of mortality in the UK, and the development of novel diagnostics and therapies remains an unmet clinical need. This project is aimed at increasing our fundamental understanding of the causes of cancer and the development of experimental preclinical models of human cancer that will accelerate the translation of novel therapies into the clinic. It is now clear that genetic status is fundamental to altered predisposition to cancer, cancer progression and response to therapy. Understanding the biological mechanisms associated with these genetic changes will be vital to facilitate new treatment strategies for human disease. In this project we wish to approach these subjects using existing strains of GA mice in novel combinations and also generate new GA mice to investigate the importance of certain genes during cancer in vivo. This will allow us to achieve three primary goals: Firstly to understand the function of certain genes during cancer with regards to how they regulate important cellular functions such as growth, cell death and migration, secondly to generate more accurate mouse models of cancer to better mimic human disease, and thirdly to use the tools and knowledge obtained in the first two aims to identify and test novel therapeutic strategies and targets.

A retrospective assessment of these aims will be due by 18 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

This project aims to investigate the function of genes implicated in cancer, for example observed in high levels in tumours compared to surrounding normal tissue. This genetic information provides a powerful tool to understand the requirement for a particular gene during cancer and therefore identifies if it will be an attractive target for therapy. For example, if we identify that gene X is up in cancer and then develop a new GA mouse to delete that gene which results in smaller tumours this would lead us to then try and inhibit gene X by a therapeutic strategy using drugs. This is particularly relevant to the treatment of cancer, as at present there are relatively few new options available in the clinic to modulate the course of disease, and therefore new, effective therapies are desperately required. We furthermore hope to gain an understanding of the degree by which genetic status determines the response to a range of therapies. The majority of clinical benefits are likely to be long term; our principal hope is that by identifying candidate genes and candidate genetic pathways we will be able to refine and accelerate drug development for human therapy. There will also be short term benefits, primarily from the testing of novel drug therapies within our models. This latter approach has the potential to directly and immediately modify clinical practice. Finally, the development of new, more accurate mouse models of cancer will provide a powerful research tool for other scientists to directly inform clinical trials and accelerate translating our findings in animal models into the clinic.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Mice are the species to be used. Mouse numbers have been extrapolated from the current funding awards held. Funding bodies require full power calculations to justify mouse numbers and sometimes compulsory use of the NC3Rs experimental design system, and thus the numbers are as accurate as possible and we estimate that we will require 21,500 mice over the 5 year period of this PPL.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

During the project, we will generate and analyse mice with both increased and reduced predisposition to cancer. This will involve the use of transgenic procedures to modify the genetic status of mice. The techniques we will use are such that we can control genetic status through exposure to chemicals or through exposure to viruses. We will also use these GA mice which are predisposed to develop cancer to model anti-cancer therapy in

the clinic. This will be achieved by exposing mice to therapeutic agents and monitoring the response of normal and tumour tissues to these therapies. Monitoring will be performed both by live imaging mice and by analysing tissue samples at death. The adverse effects will therefore be the development of cancers and the negative side effects of anti-cancer therapies. In terms of severity, these will be mostly moderate. All animals will be killed at the experimental end point.

A retrospective assessment of these predicted harms will be due by 18 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

We aim to investigate the genetic control of cancer development and the responses of cancers to a range of agents including therapies. The extremely complex nature of such *in vivo* responses makes it impossible for these studies to be completely recreated in artificial systems in vitro. However, we have been pursuing the establishment of alternative procedures that have the potential to at least partially replace the use of animals. Most prominently we have been developing 3-dimensional culture systems for both normal tissues and tumour counterparts. To date we have established these for gastrointestinal tissues and have begun to develop this approach for prostate tissues. Where possible and appropriate, we are using these *in vitro* approaches to inform our *in vivo* studies, with a view to replacing some in vivo studies and reducing and refining others, for example by establishing more precise hypotheses which can be directly tested in vivo.

A retrospective assessment of replacement will be due by 18 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

Our studies make use of the known available genetic models of disease and novel highly specific genetic models. By using such precise genetic models, we have enhanced the specificity of experiments permitting us to investigate precise genetic pathways and mechanisms. This reduces the overall numbers of animals required. We will also continue to introduce methodologies, such as novel imaging technologies, to increase the amount of data obtained from single animals and therefore reduce total animal requirement.

A retrospective assessment of reduction will be due by 18 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The approaches we will use are fundamentally a refinement of using mice, as the creation of appropriate precise genetic models ensures that the work carried out will have the most accurate and useful outcome. We will also derive multiple data from a single animal , principally by the use of in vivo imaging to allow tumour progression to be monitored over time within the same animal - so reducing the requirement for multiple timepoints and thus animal usage. To minimise welfare costs to the animals we will monitor and examine animals on a daily basis and respond to any change in health status as appropriate. We will also adopt techniques wherever possible to minimise physical intervention. For example, we will maximise the use of genetic approaches that do not require chemical injections to induce the desired genetic change and we will use non-invasive approaches (such as imaging) wherever possible. To attempt to avoid single housing of males, every attempt will be made to biopsy animals at 2 week, in order to group house at weaning.

A retrospective assessment of refinement will be due by 18 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

24. Investigation of influenza virus and avian REDACTED disease

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
- Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

No answer provided

Animal types Life stages	Life stages
Embryonic forms: Avian embryonated chicken, duck, turkey eggs	embryo, juvenile, adult
Pigs	juvenile, adult
Cattle	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The aim of this licence is to improve animal health and welfare by researching influenza and REDACTED viruses in order to limit the impact of disease. Research will focus on areas including detection of infection, characterising viruses of concern e.g. highly pathogenic strains, identifying risk pathways and developing intervention strategies.

A retrospective assessment of these aims will be due by 30 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?

- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Animal influenza and REDACTED viruses can have severe impacts on animal health and on agriculture, food security and the economy. It is particularly important to research viruses that cause outbreaks of notifiable avian disease, such as highly pathogenic avian influenza (Bird Flu) and Newcastle Disease, caused by certain strains of REDACTED. The research to gain further understanding of how these viruses cause disease and therefore develop mitigation strategies, is underpinned by this licence. Other animal influenza viruses, notably those infecting swine (Swine Flu), may also have the capacity to infect humans, and so can have a Public Heath impact. Indeed, pandemic influenza is included in the UK National Risk register of Civil emergencies.

This licence therefore provides the essential capability to safeguard the UK by enabling research into important viral diseases that have potentially significant risks including the ability to cause notifiable disease outbreaks in agricultural species and pandemics in humans.

What outputs do you think you will see at the end of this project?

This project licence will promote an improved understanding of influenza and REDACTED viruses. These viruses are known to adapt and evolve in nature with the result that the diseases they cause can alter in different hosts and cause sporadic outbreaks. Of particular concern are emergent viruses that are associated with increased disease in livestock or risk to animals and /or humans. Research questions to be addressed will include the pathogenesis and transmission of virus infections in susceptible host species, drivers of increased virus virulence and strategies to limit the impact of disease, including the use of vaccines.

The research programme will be used to address knowledge gaps ‘known unknowns’ that are of concern to stakeholders e.g. the mechanisms and drivers of virus diversification and assessment of new risk factors e.g. vaccine escape.

New information will be disseminated in the form of research papers and presentations, or may be provided as expert reports to stakeholders, the agricultural sector or other external organisations REDACTED. Data obtained will also be used to underpin risk and epidemiology assessments and will thereby contribute to mitigating disease risk by informing veterinary disease control or intervention strategies. The work conducted under this licence will also potentially provide evidence on which to base future research and diagnostic strategies.

In some cases, this work will require a dynamic, rapid response during the active phase of a disease occurrence, to address issues of national and international importance for veterinary and public health.

The study questions may be ‘unknown unknowns’ requiring reactive data generation in order to support timely disease interventions to help reduce the impact and dissemination of disease, thereby benefiting livestock, the agricultural sector and society.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

This licence supports the programme of work delivered by the group in accordance with the objectives of the establishment. The activities and impacts of this work will directly benefit the health and welfare of livestock species, through providing research outputs to assess approaches to mitigating influenza and avian orthoavula- virus diseases through improved prevention, detection and mitigation strategies.

Consequently, this work will also support of the wider national and international animal and public health communities by providing data to support policy and risk assessments. These beneficiaries include departments, organisations, governments and competent authorities. The work will also ultimately benefit trade and food security, the economy, taxpayers, consumers and society in general.

How will you maximise the outputs of your work?

The outputs of this work can be maximised through participation in collaborative studies with external organisations. As detailed, the establishment already has representation on several organisations and also have joint-funded projects that are active or in negotiation. This allows co-ordination of research efforts and good use of animals. Research findings are presented at external meetings and published.

The establishment ensures dissemination of knowledge to stakeholders, REDACTED. In the case of notifiable disease or increased risk information is disseminated through expert reports and disease risk assessments.

The establishment ensure active engagement with stakeholders, for example through the UK species expert groups and through the organisations evidence and policy teams.

Species and numbers of animals expected to be used

- Domestic fowl: No answer provided
- Other birds: No answer provided
- Pigs: 650
- Cattle: 45

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

The majority of procedures to be done are blood and swab sampling as well as inoculation of virus or immunological reagents (e.g. vaccines or antigens) by injection or instillation. Some studies will require introducing animals into a virus-contaminated environment or co-housing of healthy animals with infected animals (e.g. to assess virus transmission and infectivity in the environment).

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

The main potential adverse effects are the clinical signs resulting from infection. Often the subject of study is a novel virus strain, and the expected adverse effects are unknown. Clinical score sheets are applied to ensure humane endpoints and minimise suffering. Transient mild adverse effects may also be experienced as a result of restraint, during sampling or inoculation. Anaesthesia will be applied if anaesthesia is not more severe than the procedure itself.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Chickens
Mild 65-70%
Moderate 10-15%
Severe 20-25%

Ducks
Mild 85-90%
Moderate 5-10%
Severe 1-5%

Turkeys
Mild 45-50%
Moderate 25-30%
Severe 25-30%

Gamebirds (Pheasants and Partridges)
Mild 60-65%
Moderate 1-5%
Severe 35-40%

Pigs
Mild 98%
Moderate <2%

Cattle
Mild 99%
Moderate <1%

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 30 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Only selected viruses of scientific importance will be selected for full assessment in animals. A complete biological system is frequently required to study the course of clinical disease and the whole body response to infection. For example, the mechanisms of virus transmission from one animal to the next and disease interventions such as protective immunity from vaccination cannot be studied in non- animal alternatives. Non-sentient embryonic forms e.g. embryonated avian eggs, will be used where possible.

What was your strategy for searching for non-animal alternatives?

In vivo studies are conducted when it is necessary to answer complex scientific questions relating to infection dynamics in the biologically relevant host. For these studies, a whole animal response to virus infection needs to be assessed eg the host's immune response to virus infection, virus virulence in the host species as well as virus shedding and transmission. For studies where it is appropriate to do so, we do use continuous cell line cultures or organ tissue cultures. For the studies conducted in animals under this project licence, however, the nature of the hypotheses prevents non-animal alternatives.

Why were they not suitable?

These alternatives are suitable to address some, but not all research questions. Alternatives to animals cannot be used, for example, to address questions such as virus-host interactions, mechanisms of disease induction by a virus (pathogenesis) or vaccine efficacy.

Modelling of risk pathways in agricultural settings e.g. in poultry barn or free-range housing and assessment of risk or mitigating approaches also requires live animals.

A retrospective assessment of replacement will be due by 30 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers of animals in each research area have been estimated based on previous research programmes and project licences. These estimates considered of the number of virus strains studied, the outbreak incidents researched and the research commissioned to address scientific and stakeholder concerns. THe study outcomes will be used to inform the scientific and policy communities on potential changes and improvements to control strategies in the field. The estimates in this licence are projections to cover future disease outbreak scenarios and the anticipated research required. Numbers of animals estimated per research area also include animals that are needed for pre-screening to ensure appropriate for enrollment on a study. If not enrolled, they will be used in another protocol or another licence.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

The use of a statistically valid minimum number of animals per study will be determined based on expert advice from a professional Biostatistician. Animal studies will be designed in a consistent manner so that inter-study comparisons and data analysis can be performed.

What other measures apart from good experimental design will you use to minimise numbers?

Animal studies will be designed to maximize collection of biological materials and, where feasible, run in parallel. This will potentially reduce the number of control groups required and therefore increase the data output and research questions that can be addressed.

A retrospective assessment of reduction will be due by 30 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

This programme does not use animal models. Viruses are studied in the biologically and agriculturally relevant hosts.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Where possible, less sentient forms are used e.g. avian embryonated eggs that have been incubated less than 48h before hatch and are defined as not being sentient. In studies involving study of virus-host interactions, it is not possible to use less sentient animals as study requires use of the biologically and agriculturally relevant host.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

The species chosen are those for which the disease is most relevant in the field. Avian viruses are studied in the most relevant bird species, and swine influenza viruses in pigs as the biologically relevant host. Pilot experiments are used to refine protocols e.g. dose, route and timeline of infection required to establish infection and transmission. The Establishment strives to continually improve clinical score systems and environmental enrichment.

All species have their own specific and disease-relevant clinical observation criteria and score sheets. No animal will be allowed to progress beyond the described humane end point using a 2-3 times daily monitoring system. On site veterinary teams and animal welfare officers (NVS and NACWO qualified) participate in each study. Clinical signs serve as study endpoints when the scientific objective does not require progression of disease.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Best practice guidance is obtained from NC3Rs, ARRIVE, IAT and the RSPCA. Publications and articles are also reviewed during the approval process prior to each individual study. Where specialist training is required, inter-institutional exchanges and training visits are organised.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

The Establishment is a signatory to the NC3Rs and applies the Culture of Care in animal studies as well as the ARRIVE guidelines. Staff from the Establishment frequently attend or organise external symposia on laboratory animal welfare e.g. RSPCA and IAT meetings. Staff attending these meetings provide meeting feedback reports locally. In addition, the Establishment has a Species Group Care and Use Committee where all PILs are invited to attend. Specialist topics are presented and refinements, such as environmental enrichment, are communicated and opportunities are used for implementation. In addition, specialist knowledge exchange is organised by field and lab exchanges with other organisations e.g. PHE and Universities.

Explain the choice of species and the related life stages

The species used are the biologically relevant hosts appropriate for the viruses being studied. Where possible, an alternative to live animals is sought, for example cell or organ culture. Also, where possible, non-sentient embryonic forms (avian embryonated eggs) are used. However, for certain scientific questions where complex virus-host interactions need to be studied, it is not possible to replace a live animal. For example vaccine efficacy investigations require study of the complex interactions between virus and the full repertoire of a host's immune system.

A retrospective assessment of refinement will be due by 30 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

25. Investigation of pathways regulating tumour progression and regression

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, pregnant, embryo, neonate, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

To understand the cellular processes that contribute to cancer and identify components within these processes that might be potential targets for therapeutic intervention.

A retrospective assessment of these aims will be due by 18 November 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Despite dramatic improvements in the treatment of some cancers, many remain stubbornly refractory and even those that initially respond frequently relapse. Relapse generally results from evolutionary selection of subclonal populations of tumour cells that carry mutations conferring drug resistance or by adaptive compensatory rewiring of functional redundant intracellular pathways. The ability to interrogate simultaneously individual signalling pathways and model the evolutionary trajectory of tumours from benign mutant pre-cancerous cells to a malignant and aggressive cellular mass capable of rapid and continual evolution will be invaluable for the development of novel cancer therapies, preventative strategies and early detection.

What outputs do you think you will see at the end of this project?

Advances in fundamental knowledge

Cancer is a disease that arises because of accumulating errors (mutations) in the genetic instructions that regulate and control how the cells in our tissues replicate, spread and die. In normal tissues the processes of cell gain and cell loss are maintained in an exquisite balance: cells only replicate in the right place, at the right rate and at the right time. The process is controlled by genes that promote cell increase (oncogenes) and genes that prevent it (tumour suppressors) – these act as, respectively, accelerators and brakes. Mutations in many different oncogenes and tumour suppressors contribute to the different kinds of human cancers. Moreover, because these mutations occur and accumulate randomly, each cancer is different from every other and even within the cancer of a single patient there are many genetic variants. It is this complexity and diversity of human cancers that confounds our ability to contain and treat the disease.

However, just because cancers harbour many differences from each other does not necessarily mean that they are functionally different. Although a particular make of car may have many drivers, all those drives operate the same, common, engine. Our research aims to explore the provocative idea that certain underlying processes (engines) are shared across many, perhaps all cancers even though the mutations (drivers) that power those engines are different from patient to patient. In this regard, the huge number and diversity of cancer drivers are a distraction. Instead, our aim is to identify the common cancer engines, determine what they do and how they work, and ascertain the therapeutic benefit of targeting them with drugs. Our ultimate goal is to foster the development general anti-cancer treatment strategies that may be administered to patients irrespective of what type of cancer they have.

Our hypothesis is unorthodox and counters the prevailing dogma that cancers are irreducibly complicated. It is also backed by several decades of our research work that has identified aberrations in the function of two pro-cancer oncogenes – a molecular switch called Ras and a molecular regulator of genes called Myc – and in one pivotal tumour suppressor – a stress and damage sensor called p53 – as common to many, perhaps all human cancers. However, none of these is yet targetable by drugs. Hence, our only means for deciphering the roles of these cancer engines is to use sophisticated, switchable mouse genetic models. These models allow us to reversibly switch Myc, Ras and p53 on and off in normal and neoplastic tissues and thereby directly determine what the roles of these engines are in the genesis and maintenance of different cancer types. Over the past few years, our principal focus has been on the Myc "engine," whose diverse activities appear to be absolutely fundamental to the genesis and maintenance of diverse cancers. We have painstakingly identified and mapped the web of interactions that link Myc activity to control of the tumour itself, as well as the pathogenic inflammatory and

immune-suppressed tissue that surrounds and supports the tumourin its midst. Our mouse models also allow us to assess the therapeutic consequences of manipulating such common cancer engines: would interfering with their function be therapeutically effective? If so, why? And how toxic might the side effects of such interference be for a cancer patient. In this way, we will establish in principle what are the most effective and tumour-specific cancer therapeutic targets, so informing pharmaceutical strategies for future cancer therapies. We will also test existing therapies in these same controlled and reproducible disease models. The data so generated will be used by chemical engineers to guide the design of completely new drugs of general applicability across a wide range of neoplastic diseases.

Production of valuable resources

We have developed and validated some of the core regulatable genetically altered mice that we will utilize in our future studies. Additional novel mouse strains will be developed and used in conjunction with existing strains and with those available to us from commercial sources or from collaborators. Given our proven track record in the development of novel switchable genetics in mice, we are highly likely to generate significant results. Our results will be published in influential peer-reviewed journals and disseminated through scientific seminars. Novel regulatable genetically altered animals developed during the course of this project will be made freely available to other researchers interested in the functions of the target proteins in normal adult tissue and in cancer and other diseases.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

It has always been our strict policy and practice to freely distribute all data and reagents, including genetically modified mouse strains, without conditions and prior to publication. This policy will also apply to scientific outputs covered by this project. We are investigating the possibility of depositing our mouse strains with the European Mouse Mutant Archive (EMMA/Infrafrontier). There will be no moratorium on presenting our research at national and international scientific meetings and via bioRxiv (https://www.biorxiv.org) prior to submission to open access peer-reviewed journals.

Sequence data will be maintained by the host institution and, in addition, RNAseq and ChIPseq data will be submitted to publicly accessible databases such as ArrayExpress (http://www.ebi.ac.uk/arrayexpress/). Other data such as mouse strain, husbandry and genotyping are stored on local databases but relevant information will be made available in suitable formats on request.

Where data are not available through public databases, interested parties will be provided with a secure digital links to the requested data via the host institution.

In the longer term, our hope is to stimulate increased academic, biotech and pharma efforts towards inhibiting the Myc oncogene and identify Myc effectors, crucial for initiation and maintenance of tumours, that are potential therapeutic targets.

How will you maximise the outputs of your work?

All our research will be routinely presented at national and international scientific meetings and via bioRxiv (https://www.biorxiv.org) prior to submission to open access peer-reviewed

journals. Our group participates in many collaborative endeavours, some of which use mouse models developed under our existing PPL. In addition, the applicant and other lab members are frequently invited to present at scientific meetings throughout the year at which expertise on the animal models is freely communicated.

Species and numbers of animals expected to be used

- Mice: 41000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Typically, non-toxic gene/protein activating agents will be administered to genetically altered mice, either in drinking water or in the diet. In a minority of cases such agents will be administered by gavage or injection (usually intraperitoneal). Administration of such substances induces tumour development. In some cases, a potential therapeutic compound is administered at the same time to determine its effect on tumour development. In addition, a small number of mice spontaneously develop tumours after several weeks/months. Our experiments last a few days/weeks and we aim to limit the tumour size to minimise suffering prior to switching off the activity of the gene/protein and/or administering a therapeutic agent to assess tumour regression. One important hypothesis, for which we already have convincing preliminary evidence, is that tissue-specific programmes that instruct tissue regeneration and resolution in response to injury are hijacked by oncogenic proteins. Thus, some animals will be subject to tissue-specific injury to understand these processes and how oncogenic proteins are involved. For example, liver is the principal organ involved in de-toxification of hazardous compounds and, as a consequence, has evolved a remarkable capacity to regenerate. Continuous chronic assault on liver from hepatotoxic agents such as alcohol, mycotoxins or natural alkaloids drives a sustained cycle of damage and repair that is thought to contribute both to liver failure and liver cancer. We will use controlled, sub-lethal doses of CCl4 to elicit acute liver injury and then monitor the roles of Myc and Ras oncogenic signalling pathways in instructing the rapid repair and regeneration of the organ. Pancreas is another tissue of interest where overlapping molecular programmes appear to underpin both repair of pancreatic injury and pancreatic adenocarcinoma, a cancer with dismal prognosis. Acute or chronic injury in pancreas will be induced by intraperitoneal injections of cerulein, a cholecystokinin (CCK) analogue that enhances secretion of digestive enzymes from the pancreas acinar cell and acutely induces mild to moderate acute interstitial pancreatitis. Recovery from each cerulein dose is rapid and involves regeneration and remodelling of pancreatic exocrine tissue that transiently shares great mechanistic overlap with pancreatic adenocarcinoma. Similarly, naphthalene is rapidly metabolised into a number of reactive epoxide and quinone metabolites by lung epithelium-specific cytochrome P450s (CYP2A5 and CYP2F2) and thereby induces acute lung injury that triggers rapid regeneration of the damaged lung epithelium. In all these instances, injury is transient, rapidly repaired, and all mice recover within a few days. At the end of the experiment the mice are humanely killed and molecular analyses conducted on multiple tissues.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Our experimental models are designed to induce tumours in lung, pancreas, skin, breast and B cells or tissue damage in lung, pancreas and liver of mice that will cause the least suffering for the shortest period. Nonetheless, the nature of the experiments (tumourigenesis and tissue injury) will cause inevitable adverse effects and mice may suffer transient weight loss, tumour development (which in the lung may lead to respiratory distress) and pain (largely confined to pancreatic tissue injury).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Our aim is to minimize animal suffering commensurate with directed scientific experimental design and statistically valid output. Indeed, we would argue that animals experiencing persistent pain are not a good experimental model. Nonetheless, our experimental models are designed to induce either tumours in lung, pancreas, skin, breast and B cells or tissue damage in lung, pancreas or liver of mice, and this will likely cause at least transient suffering. Since the majority of our genetically altered animals harbour switchable alleles, we can accurately and reproducibly switch on (and off) key proteins that govern tissue regeneration/tumour progression and wound resolution/tumour regression, allowing us unprecedented accuracy in predicting the kinetics of tumour growth (and regression). This allows us to either kill the animal before severe adverse effects are manifest or initiate tumour regression and thereby forestall any adverse impact. The majority of animals, such as the 60% used for breeding, will not exceed mild severity. Experimental animals will experience moderate severity (20-30%) and some animals (<2%) may experience severe adverse effects. For experiments designed to induce tissue damage in a single organ (lung, pancreas, liver) we aim, via reductions in dose, to induce the minimum damage that initiates a regenerative response. The damage inflicted is acute, tightly controlled and transient and the mice recover within a few days. Lung damage: we intend to administer naphthalene by inhalation (West et al. 2001. Toxicol Appl Pharmacol 173, 114-119) rather than injection and will seek local expertise on this method. Pancreas damage: cerulein is a cholecystokinin (CCK) analogue that enhances secretion of digestive enzymes from the pancreas that induce a form of self-injury. Cerulein-induced damage to the pancreas may be acute or chronic and, while widely used to cause pancreatic damage and induce pancreas inflammation and injury (akin to the human condition pancreatitis), the effects on the tissue and adverse effects to the animals can be variable and a small percentage (<10%) of animals may experience severe effects for short periods (before being killed). Toxin-dependent liver damage causes short-lived moderate adverse effects and the liver very rapidly recovers both function and fom.

What will happen to the animals at the end of the study?

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 18 November 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The aim of this project is to elucidate the contribution of Myc to tumourigenesis, tumour maintenance and normal physiology and to validate Myc as a potential therapeutic target for cancer. Since in vitro studies cannot adequately recapitulate the physiological and systemic context and interactions in which cancers evolve it is necessary to employ animal models. For example, interactions between the tumour cells and several different types of stromal cells that constitute the "tumour microenvironment" are crucial determinants in tumour evolution, growth and survival. Inhibition of the soluble paracrine signals that inhibit these interactions is a promising basis for tumour therapy whose impact and tumour dependence can only be investigated in the whole organism, particularly since some of the key stromal cell types (i.e. lymphoid and inflammatory cells) are recruited to the developing tumour from distant organs.

What was your strategy for searching for non-animal alternatives?

Where possible, our animal studies are replaced and/or complemented by cell/tissue/organoid culture experiments using both commercially available and mouse-derived established cell isolates. These studies are invaluable in investigating the cell autonomous nature of cell signalling and cell processes but cannot address complex interactions between multiple cell types. We will consider the use of 3D organoid models that have been developed for lung, pancreas and liver (three prominent tissues in our studies) but these still fail to address the inherent difficulties of modelling complex and highly tissue- specific interactions between tumour and normal cells that we know is critical to cancer growth and response to therapy *in vivo*.

Why were they not suitable?

Cells in culture are subject to an environment very different from that *in vivo*. They experience a variable and abnormal oxygen tension that is only poorly controlled and defined, are usually cultured in a vast excess of glucose and ill-defined mitogenic growth factors, survival factors, cytokines and chemokines of (typically) bovine origin. Their rapid proliferation in culture is abnormal and facilitates rapid evolutionary selection (e.g. for cells that proliferate faster and/or are more resistant to cell death). Although organoid cultures offer some improvements, they are severely limited by the rather crude and unrepresentative matrices (e.g. Matrigel) that must be used and which do not reconstruct the complex interaction of multiple cell types. For example, a functional dynamic immune and inflammatory system that responds to, and is instructed by, the tumour cells - a central mechanistic tenet of our research - cannot be reconstituted *in vitro*.

A retrospective assessment of replacement will be due by 18 November 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The aim of this project is to elucidate the contribution of Myc to tumourigenesis, tumour maintenance and normal physiology and to validate Myc as a potential therapeutic target for cancer. We will use well- validated mouse models with which we have extensive experience. There is considerable overlap in our experimental design that serves to reduce total numbers of animals used.

Mice will be randomly allocated to experimental groups, maintaining a comparable segregation of age, size and gender. All animals will be maintained in the same environment. Details of the random allocation will be retained by our existing chief animal technician, who is not directly involved in this project. The same person will administer experimental agents (or control substances) and retain a key to identify recipient mice. This key will only be accessed after analysis has been completed .

For most experiments we calculate that 5 - 6 animals are required per group to generate biologically meaningful and statistically valid data. This is based on a range of considerations agreed with our advisory biostatistician.

Since most of our studies are based on the dynamic changes that follow Myc activation, we will generally require 3 time points per experiment (typically, but not exclusively 1, 3 and 7 days) making a total of 30 animals per experiment. In this case the choice of the test of interest might be to compare with and without treatment, at different time points, and across time points (all treated). Again, all known statistical considerations will be implemented to be sure to derive the maximum usable data from each experiment.

Since many of our experiments require animals with complex genetic makeups, we will carefully plan breeding strategies to minimize the number of animals of incorrect genotype. For example, some experiments require animals with a specific combination of 5 or more alleles. Hence, more than 65% of the animals will be involved in breeding protocols only.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Calculations are informed by reference to Festing, MF and Altman, DG, 2002 (ILAR J 43, 244-258) and NC3Rs Experimental Design Assistant (https://eda.nc3rs.org.uk/eda/landing) and with assistance from the in-house statistician dedicated to optimising use of animals in experiments.

What other measures apart from good experimental design will you use to minimise numbers?

Since most of our experimental animals have complex genotypes we have carefully planned breeding strategies to maximize the number of suitable experimental animals and control littermates. All animals will be humanely destroyed at the end of experiments and tissue samples taken for further experiments. Where possible (eg where tissues can be used as controls for other experiments) mouse tissues will be shared amongst the research group. This will maximize the amount of information that can be acquired from the minimum number of animals. Where appropriate pilot studies will be conducted to determine feasibility and efficacy.

A retrospective assessment of reduction will be due by 18 November 2025

The PPL holder will be required to disclose:

How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

One of the problems with classical transgenic and xenograft mouse cancer models is the unpredictable and highly variable time it takes for tumours to emerge. In such instances, it is unknown why some animals take longer to exhibit disease than others. For this reason, all animals have to be constantly monitored and their state of health inferred indirectly by external signs. Such unpredictable variability makes minimisation and mitigation of adverse effects much more difficult. The great variability in the time to tumour presentation in such classical mouse models also means that the critical events that eventually drive overt outgrowth of tumours, and timing and sequence of such events, are very poorly defined, making it almost impossible to establish the cause-and-effect events that cause the cancer.

By contrast, our sophisticated switchable genetic technologies allow us to regulate expressionor activity of key cancer genes at will, in real time in target mouse tissues. This triggers tumour formation and regression with highly reproducible, consistent, predictable and rapid kinetics. This allows detailed assignment of which, when and how the various oncogenic events accumulate to cause cancers while greatly reducing numbers of animals needed in each cohort to achieve statistically valid data. Moreover, because of the highly predictable latency and rate at which our models develop tumours, we are able to terminate most experiments at a very early, incipient, stage when tumours are small and have not yet spread or metastasised. Consequently, most mice exhibit only moderate

adverse effects and the small number that perhaps exhibit severe effects can be killed immediately.

Our studies on the role played by the Myc protein in tissue regeneration, and its relationship to cancer, requires animals in which different single tissues are deliberately damaged. Although not causing lasting harm (the tissues rapidly and efficiently regenerate), these models may generate transient suffering and pain. Where possible the pain is mitigated by analgesics.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Our scientific goals rely on being able to model the development (and regression) of adult human cancers in the mouse. Thus, it is not possible to use more immature stages or terminally anaesthetised animals. Mouse physiology is sufficiently similar to that of humans to generate passable representations of the human disease. This is facilitated by detailed knowledge and comparison of mouse physiology and genetics. Tumourigenesis is a dynamic process, taking several years in humans - in the mouse, we can speed up and precisely regulate this process using a number of genetic manoeuvres such as reversibly switchable protein activity and/or gene expression.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

We will continue to use rapidly switchable models that allow short-term, consistent and predictable outcomes that allow us to limit welfare costs to the animals.

For example, in some experiments we employ the commonly used pdx1-Cre allele to activate expression of oncogenic Ras and Myc proteins specifically in the pancreas. However, we have observed extra-pancreatic expression in this mouse model leading to collateral adverse effects, specifically hyperplasia and neoplasia in the intestine. For this reason, we developed a replacement mouse model in which expression of Ras and Myc is tightly restricted to the pancreas and extra- pancreatic adverse effects do not occur.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We adhere to the guidelines published in Workman *et al*. (2010) Guidelines for the welfare and use of animals in cancer research. BJC 102, 1555 - 1577 and the NC3Rs ARRIVE guidelines.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We are advised of advances in the 3Rs via regular correspondence (email). Furthermore, our dedicated technician attends relevant 3Rs - sponsored meetings. Information is disseminated to the rest of the group. REDACTED

Explain the choice of species and the related life stages

The aim of this project is to elucidate the contribution of the Myc protein to tumourigenesis, tumour maintenance and normal physiology and to validate Myc as a potential therapeutic

target for cancer. Since cells cultured *in vitro* do not adequately recapitulate the cellular context and interactions in which cancers evolve it is necessary to employ animal models. For example, interactions between the tumour cells and several different types of non-tumour (stromal) cells that constitute the "tumour microenvironment" are crucial for tumour growth and survival. Inhibition of the soluble signals that drive these interactions is a promising basis for tumour therapy that can only be elucidated in the whole organism, particularly since some of the stromal cell types recruited to the developing tumour originate in distant organs. All of the proposed animal experiments involve mice. The mouse is the most suitable model system in which to perform these studies. Our major interest is in adult human tumours (particularly those arising in the lung, pancreas and liver) and our experimental mouse models generate tumours in these organs of adult mice that closely resemble the human disease. Moreover, the mouse genome is well characterized and can be manipulated by gene targeting and there is extensive knowledge on the breeding and husbandry of rodents. The animals will be maintained in Home Office approved facilities offering rigorous guidelines to ensure the best welfare and so that animal numbers are kept to a minimum.

A retrospective assessment of refinement will be due by 18 November 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

26. Investigation of vitamin D biology in companion animals

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

No answer provided

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The important role vitamin D plays in maintaining healthy bones has been known for nearly a century. However, there is increasing interest in the potential health benefits of vitamin D beyond the skeleton in both humans and animals.

My research group studies the role vitamin D plays in maintaining and regulating the health of dogs. Our previous research has found that vitamin D status of dogs is dramatically altered in numerous diseases and we now wish to understand more about why this happens and then define the importance of these changes.

In particular, we are keen to establish whether vitamin D influences the immune system of dogs. Our previous research in mice has shown that vitamin D metabolites can dramatically alter the nature and behaviour of immune cells. Importantly, we found that high doses of active vitamin D metabolites can block the development of inflammation in the brain following the activation of immune cells.
Consequently, we now wish to examine whether vitamin D can alter the immune response in healthy dogs.

We also wish to examine how vitamin D is metabolised in healthy dogs in order to understand how commonly used treatments, such as administration of fluids directly into the vein, can influence vitamin D status.

We also intend to understand more about vitamin D biology in patients who attend our hospital with diseases that develop spontaneously. We are keen to study diseases which develop naturally in patients to avoid the need to induce disease in healthy dogs. In addition, induced diseased in healthy dogs is unlikely to mimic all aspects of the spontaneous diseases we diagnose in our clinics. In particular, we wish to examine why dogs with severe gastrointestinal diseases typically have very low vitamin D status and whether this causes ill health in body organs beyond the intestines. In addition, we want to define how best to treat dogs with intestinal disease in order to improve treatment outcomes.

Retrospective assessment

Published: 16 May 2023

Is there a plan for this work to continue under another licence?

No

Did the project achieve its aims and if not, why not?

The project was revoked without any patient being enrolled onto the proposed studies. This was because the principal investigator changed job.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

A deeper understanding of vitamin D biology in dogs is likely to lead to development of superior treatments for client owned dogs with spontaneous illnesses. In addition, a better understanding of vitamin D homeostasis in dogs may offer a better understanding of the important diseases in humans.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We will use 56 dogs in our studies.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

The procedures undertaken in this study will include blood sampling, diagnostic imaging. The expected adverse effects will be limited to, and not be noticeably more, than the standard diagnostic tests and normal treatment for the conditions and will be managed by an experienced team of onsite veterinary specialists

Adverse events may include minor skin bruising from site of blood sampling. Other less common potential adverse events include high concentrations of calcium following vitamin D administration. This is very unlikely given the dose of vitamin D which we will use in our studies. Other potential adverse events include administration of intravenous fluids, extended anaesthesia times and exposure to diagnostic radiation. All of these are standard clinical procedures with a very low level of adverse events. If any adverse events occur we are ideally placed in our multidisciplinary hospital to deal with any eventualities.

Retrospective assessment

Published: 16 May 2023

What harms were caused to the animals, how severe were those harms and how many animals were affected?

The project was revoked without any patient being enrolled onto the proposed studies. This was because the principal investigator changed job

Replacement

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

Our aim is to better understand vitamin D biology in dogs. We believe this approach is the epitome of best practice on compliance with the 3Rs since we are studying disease processes which have already developed rather than inducing them in otherwise healthy animals.

There are no pre-existing datasets which allow us to deliver our proposed programme of work. There are no non-animal approaches which allow us to understand how vitamin D modulates health in dogs.

Retrospective assessment

Published: 16 May 2023

What, if any, non-animal alternatives were used or explored after the project started, how effective were they and are there any lessons worth sharing with others?

The project was revoked without any patient being enrolled onto the proposed studies. This was because the principal investigator changed job

Reduction

Explain how you will assure the use of minimum numbers of animals.

As the animals will develop the disease spontaneously, we are avoiding the need to induce illnesses in otherwise healthy animals. Control, healthy animals will be used as controls, where appropriate, to facilitate the meaningful interpretations of data gathered from the spontaneously ill dogs. For each experiment, we will use the minimum number of animals required to address the scientific question of interest. Where there is pre-existing and relevant preliminary data, power calculations will be performed to guide the numbers of patients which should be recruited for each experiment.

Retrospective assessment

Published: 16 May 2023

How did you minimise the number of animals used on your project and is there anything others can learn from your experience?

The project was revoked without any patient being enrolled onto the proposed studies. This was because the principal investigator changed job

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Our aim is to better understand vitamin D biology in dogs. We may identify further diseases of relevance to these species that require improved scientific knowledge in order to produce effective treatments for them and thus the need to include dogs in this licence. Wherever possible, we will undertake procedures, notably blood sampling, at the same time as clinical procedures. Clinical procedures will be undertaken by a veterinary surgeon or nurse to ensure animal suffering is minimised and all procedures are done to RCVS standards.

Retrospective assessment

Published: 16 May 2023

With the knowledge you have now, could the choice of animals or models used have been improved at all? How did you minimise harm to animals during the project?

The project was revoked without any patient being enrolled onto the proposed studies. This was because the principal investigator changed job

27. Ion regulation in cardiac, skeletal and vascular smooth muscle

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants.
- Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Key words

Exercise, heart, heart failure, skeletal muscle, sodium

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Heart failure and high blood pressure go hand in hand in the elderly. Approximately 70% of men over the age of 70 show some evidence of having a large heart (hypertrophy) and almost the same proportion of men also have high blood pressure. Large hearts do not contract (or relax) properly and so this process of hypertrophy is a prelude to heart failure. These problems are not confined to men. One recently described type of heart failure is called HFpEF – this is where the heart does not relax properly between beats. HFpEF (like other forms of heart failure and high blood pressure) affects both men and women however it is particularly prevalent in post-menapausal women who have other comorbidities (ie diabetes, obesity etc). There is now a large amount of evidence suggesting that changes in ion regulation (sodium, calcium, potassium etc) inside heart and other muscle cells may contribute to hypertension, hypertrophy, heart failure and even skeletal muscle fatigue. The objective of these studies is to learn more how disease processes, ageing and gender all interact to affect outcome. Our aim is to understand more about the role of ion regulation in these diseases and to identify and develop new ways to treat heart failure, skeletal muscle fatigue and hypertension (high blood pressure).

A retrospective assessment of these aims will be due by 10 December 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The objective is to learn more about the disease process and to develop new ways to treat heart failure, skeletal muscle fatigue and hypertension (high blood pressure). We propose to test new drugs designed to treat both heart failure, hypertension and the skeletal muscle fatigue that so often accompanies the later stages of heart failure.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We propose to use rats (<1,000) and mice (<4,000) over 5 years.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Under general anaesthetic with recovery, animals will be subjected to a variety of techniques to simulate heart failure. They will be allowed to recover and then compared to ‘athletic’ animals that have been trained to run or swim predetermined distances or times. In this way we can compare disease- induced heart growth with exercise-induced heart growth. While the exercise is not severe, the induction of heart failure is and these animals will show all the signs and symptoms of human patients with heart failure – e.g. breathlessness, fatigue, listlessness etc. During the study, animals may be imaged using techniques such as echocardiography or MRI (similar to those used in people). At the end of the experiment all animals will be humanely killed and their tissue taken for further study.

A retrospective assessment of these predicted harms will be due by 10 December 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

Heart failure is a complex disease involving the whole body. Hormones, the central nervous system, the kidneys, etc all actively influence the disease progression. Athletic training also alters the whole-body – it changes our circulation, metabolism, fat, blood composition, insulin secretion, nervous system controls, hormones etc. It is impossible to simulate these complex scenarios in cells in culture or in computer models.

A retrospective assessment of replacement will be due by 10 December 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

We will use sophisticated monitoring and serial non-invasive imaging systems to maximise the data that is generated from a single animal. Studies that 25 years ago would have taken tens of animals can now be done in a single animal. All experiments are designed in advanced using statistical power analysis to ensure that the minimum number of animals will be used that is compatible with proving our scientific hypotheses. We will also, by combining tissue from one animal, be able to study the consequences of 3 distinct pathologies affecting the heart, skeletal and vascular muscle. This reduces the number of animals required by 2/3rds.

A retrospective assessment of reduction will be due by 10 December 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Many years ago such experiments used larger animals (often dogs). Recently, however, miniaturisation has allowed us to use smaller animals in particular mice and rats. The mouse has become the preferred animal of choice for many studies as it is cost effective to house in large numbers, it has a short gestation period and, in captivity, breeds readily and repeatedly just as it does in the wild. This makes it the species of choice for manipulating its genome (that is adding, deleting or mutating its genes) to test the role of specific genes or proteins. Mice also love to run! So they are a good species in which to

study exercise physiology. Given a running wheel, a mouse will voluntarily run for 4-8 km/night – our record is 17 km in one night – the equivalent of a human running to Australia and back! Running wheels and environmental enrichment enhance the environment for the animals. We are refining the swimming protocols so that the animals acclimatise to the procedure and stress is kept to a minimum. All surgical experiments are done using anaesthesia and analgesia (pain killers) as would be provided to humans and post-operative care will be designed to minimise stress and suffering. Any animal found to be suffering outside the expected limits of this application will be humanely killed.

A retrospective assessment of refinement will be due by 10 December 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

28. Mechanisms of heart regeneration in fish and mouse

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Zebra fish	adult, embryo, neonate, juvenile
Oryzias latipes (Medaka)	adult, embryo, neonate, juvenile
Mice	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

To better understand the role of metabolism, inflammation, scarring and the light/dark cycle during heart regeneration.

A retrospective assessment of these aims will be due by 08 October 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these

could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Coronary artery disease is the single most common cause of death in Europe, accounting for 1.8 million deaths in Europe each year. There are around 7.4 million people living with cardiovascular disease in the UK, more than twice as many people than with cancer and Alzheimer's disease combined and causing more than a quarter (28 per cent) of all deaths in the UK. Blockage of one of the arteries supplying blood to the heart results in a lack of oxygen in the downstream heart muscle and subsequent death of the starved cells (heart attack). The dead heart muscle is replaced by fibrous scar tissue in those fortunate enough to survive the heart attack, providing a necessary solution in replacing necrotic muscle to close the ventricular wall. However, the non-contractile fibrous tissue will never be replaced by new heart muscle, and may cause severe contractile dysfunction, resulting in heart failure and even recurring myocardial infarction. The combination of improved treatments for cardiovascular disease, increased public awareness of the risk factors, and government strategies aimed at helping people live a healthy lifestyle, has reduced mortality rates, meaning that the number of people who survive a heart attack is steadily increasing. This impressive improvement in survival rate as well as the increasingly elderly population, however, also mean more people living with the long term effects of reduced heart function and heart failure, costing the UK economy £9 billion per year, with this number steadily increasing every year.

Complete regeneration of the adult heart after injury is a feature exclusive to a limited number of species, including lower vertebrates such as the zebrafish and salamander. Injury to a zebrafish heart results in a spectacular scar-free regeneration process, with the wound tissue completely being replaced with new, functional cardiac muscle. If we can discover what fundamental mechanisms drive natural heart regeneration in fish, we can directly apply this knowledge to heal the human heart after injury. Differences in metabolism, inflammation, scarring and the light/dark cycle are thought to be important differences between animals that can regenerate their hearts and animals that cannot, but not much is known about how these processes regulate heart regeneration. If we can understand what is so unique about the fish heart that it can regenerate, we can apply this knowledge to find therapies to heal the mouse and human heart after a heart attack and during heart failure.

What outputs do you think you will see at the end of this project?

We will know more about the mechanisms underlying heart regeneration in fish and how we can use this knowledge to repair the mammalian heart after injury. We will have improved our understanding of what the difference is between heart muscle cells that can regenerate and ones that cannot, and how the immune response influences this process. Within the time frame of this licence, we aim to have identified >20 new genes that play an important role during heart regeneration and found drugs that target these genes. We will publish these results in scientific journals to help advance the field as a whole. For the most promising targets we will establish collaborations with spin out/farmaceutical companies soon after the licence as well as in the longer term.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The aim of this project is to better understand natural heart regeneration and to take our knowledge forward to induce heart repair in adult mouse hearts. During the time of this licence, this knowledge will be shared with the wider academic community, to be able to find collaborations that further drive the results forward. Our results will be shared at conferences and in papers, and discovery of new drug targets will be shared with spin out/farmaceutical companies as soon as possible, within the time frame of this licence or soon after wards, In the long term, the aim is to find therapies to help patients with heart attacks and heart failure.

How will you maximise the outputs of your work?

We will continue to collaborate locally, nationally and internationally to advance the project and to make the best use of expertise and animals. Initially with other research groups, followed by spin out/farmaceutical companies as soon as we have identified drug targets. We will present our work at national and international conferences to disseminate the new knowledge. We will publish both successful and unsuccesful approaches to help the research community and avoid duplication of work.

Genes identified from our fish experiments will be taken forward to the mammalian mouse model within the timeframe of this licence to be able to identify drug targets that work across species. Identified targets will be shared with the wider community, academic and industry, to, in the longer term, take these further to human patients.

Species and numbers of animals expected to be used

- Zebra fish: 10000
- Other fish: No answer provided
- Mice: 4000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

The typical experience for animals on this protocol, both fish and mice, is either breeding to produce offspring that can be used for surgery, or cardiac surgery followed by isolation of the heart for further analysis.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

We are breeding GA animals, with GA that affect genes that have a cardiovascular function and could result in clinical symptoms. Most GA animals are expected to have no abnormal clinical signs due to the genetic modification, but the phenotypes of new GA animals are difficult to predict. We also perform cardiac surgery that has the risk of sudden death. The majority of sudden cardiac death will be during or immediately following surgery while the fish/mice are being monitored for recovery, with a small proportion

suffering sudden death due to cardiac insufficiency which can't be predicted by clinical signs as with human patients, though increased monitoring used to mitigate. These animals will be closely monitored for signs of discomfort or distress, relating to cardiac insufficiency such as abnormal swimming activity, increased rate of respiration, and/or lack of reaction to the presence of food for fish and increased heart rate, breathlessness, inactivity and decreased feeding for mice. Any animal showing these signs will be humanely killed by schedule 1 method if these symptoms do not approve within the time frame carefully set for each species.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Sub-threshold: fish > 30%, mouse > 30%
Mild: fish <10%, mouse <10%
Moderate: fish <10%, mouse <10%
Severe: fish <50%, mouse <50%

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 08 October 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The heart is a complex organ containing many cell types of which arguably the most important are the muscle cells, responsible for the pumping function of the heart and the endothelial and smooth muscle cells, which make up the blood vessels of the heart. Many of the experiments we propose will be carried out on isolated pieces of cardiac tissue or cell cultures of heart muscle, blood vessel, and other heart cells studied in the laboratory. However cells in a test tube or in a tissue culture dish cannot be used to study the complex changes occurring in the complete heart, nor how it functions in a living animal. Especially effect of the body immune response is important for heart regeneration, for both scarring and healing. This cannot be replicated in vitro. Equally, isolated cell populations in tissue culture transform to adopt different functional characteristics, compared to the equivalent cells as they reside in the heart proper, which confounds any experiments to determine the effect of externally added factors on heart injury and repair. Despite these caveats, we perform experiments on cells, tissues or whole hearts (excised under anaesthesia) in vitro

as an integral component of the project to enable the testing the role of the targets during heart regeneration and find factors that potentially play a role in protecting the heart against periods of ischaemia and in repair/regeneration of the heart after myocardial injury. While researchers have tried to regenerate hearts in vitro, heart regeneration is a unique process that only occurs within the body. Therefore, the only way to understand how heart regeneration occurs, is to study animals that can and cannot regenerate.

What was your strategy for searching for non-animal alternatives?

Whilst we do not understand the process well enough yet to use computer modelling of heart regeneration, we have created a large database of bioinformatics data on our models to be able to carefully select targets before any in vivo experiment. We collaborate with bioinformaticians to further combine our datasets with already published datsets to find the most promising candidates for further study. We use cell culture approaches to determine the effect of over expression/gene loss-of-function on pathways to help establish mechanisms of action, before going to the in vivo models, but also alongside these experiments to help us understand the in vivo models better. We have considered culturing fish hearts in vitro to be able to study heart regeneration in more detail, but the hearts only regenerate within the body. We are setting up in vitro cell culture lines from fish cells, that will allow us to further test substances in vitro before taking the most promising candidates in vivo. This will also allow us to perform in vitro drug screens and replace animal work.

Why were they not suitable?

Our bioinformatics data sets help us to replace a large number of animals, and only test the most promising targets in vivo, but heart regeneration can not be modelled in vitro. Also the fish heart cannot regenerate in vitro. Cell culture approaches have been useful in determining cellular consequences and probing mechanisms of action and exploring therapeutic potential. However, in vitro cell-based assays cannot address the effect of our in vitro manipulations on disease initiation and progression or even regression following treatment with therapeutics.

A retrospective assessment of replacement will be due by 08 October 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The animals numbers have been established based on power calculations and our experience with these experiments. We have made a prediction based on these calculations and the expected funding over the 5 years.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

To reduce the number of animals, we use the NC3Rs' Experimental Design Assistant during the experimental design as well as carefully select the targets of interest using our bioinformatics database. Besides using power calculations to use the correct number of animals, we also regularly discuss our experiments with a local statistician. For our sham and control experiments, wherever possible we use historic controls.

What other measures apart from good experimental design will you use to minimise numbers?

We have meetings with the group as well as with collaborating groups to discuss optimal experimental design. We sit down to plan the most effective breeding strategy, especially for inducible lines that require multiple crosses. For all new targets of interest, we will first do a pilot experiment to determine if it has a function during heart regeneration before further characterisation. To reduce numbers of animals, we analyse the hearts using sections, allowing us to test about 30 different genes per heart. When available, we obtain transgenic/mutant fish from stock centres around the world. As these are established lines we only obtain minimal numbers. To generate the lines ourselves would require much larger numbers. Over the years, we have performed sham and control experiments when needed, allowing us to build up a database with control samples. Therefore, wherever possible in our experiments we will use these historic controls.

A retrospective assessment of reduction will be due by 08 October 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

We will use fish model because of their unique ability to regenerate their hearts. We use different fish species that have their specific value due to their ability to regenerate or scar. Only when we have found targets, pathways and cellular mechanisms that regulate heart regeneration in fish and are possible candidates to take further to test for therapeutic options, we will use the neonatal and adult mouse models for cardiac injury. The neonatal

mouse is comparable to fish in that their hearts regenerate and will be used to test if the targets/mechanisms we have found to regulate heart regeneration in fish also regulate heart regeneration in mammals. The adult mouse is comparable to humans and will be used with the aim to try to change their response to a more neonatal mouse or fish- like response and improve their ability for heart regeneration. For the cardiac injury method, we and other groups have carefully optimised the method to cause the least pain and distress.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

We will select targets after careful literature, database and in vitro selection and will the test the candidates of interest in the least sentient animal possible for this research, the fish, before going to the mouse model for the most promising candidates. While we can use fish to identify targets that are crucial for heart regeneration, we need to know if these targets also play a role in the mammalian (mouse) heart to know if we have found targets that could possibly used to design therapies to treat cardiovascular disease in patients.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

The disease models in this licence are established in our laboratory and that of our collaborators. We have refined protocols in collaboration with other groups in REDACTED with whom we collaborate and who themselves use these techniques. For instance, it is now standard practice that recovery surgery is performed earlier in the day to allow sufficiently frequent monitoring within normal working hours. After procedures, we will provide pain management and increase monitoring to be able to identify and treat welfare issues as soon as the appear.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We will follow the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes as well as keep up to date with literature on these techniques, which will allow us to immediately implement any further refinements in our experimental protocols. For all procedures, the LASA guiding principles for preparing for and undertaking aseptic surgery will be used.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We regularly check relevant websites, including NC3Rs, FRAME and Altweb, and NC3Rs newsletter for alternatives for the use of animals in research. We always attend the departmental animal welfare meeting for further updates on alternatives from within the community. Any alternative that we identify that is relevant for our work is discussed in our group and implemented if possible.

Explain the choice of species and the related life stages

We will use the adult zebrafish and Astyanax mexicanus surface fish because of their unique ability to regenerate their hearts. Medaka and Astyanax mexicanus cavefish cannot regenerate theirs hearts. Directly comparing heart regeneration versus scarring between

fish, especially within the same species, allows to focus on the mechanisms underlying these processes. Using different species that can or cannot regenerate will allow us to find the overarching mechanisms that is similar between the different species. We also use neonatal mice because of their unique ability to regenerate their hearts. What we learn from these models will then be tested in adult mice, that are chosen for their inability to regenerate.

A retrospective assessment of refinement will be due by 08 October 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

29. Modulators of Immunity and Inflammation

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, neonate, juvenile, pregnant, embryo
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Research into parasite infections with two overarching objectives, (i) to promote protective immunity through vaccination and/or other interventions; and (ii) to exploit natural immunomodulating products of parasites in the therapy of inflammatory disorders.

A retrospective assessment of these aims will be due by 13 November 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

More than 2 billion humans worldwide are afflicted with parasitic helminth infections, which are also highly prevalent in livestock with substantial welfare and economic consequences in the UK. No vaccines are currently available for human use, and are very limited in the veterinary sphere. In addition, a high percentage of the human population, particularly in countries such as the UK, suffer from inflammatory disorders (such as asthma, diabetes, inflammatory bowel disease and multiple sclerosis) which are poorly treated. Helminth (worm) parasites have evolved natural pathways to suppress inflammation and their molecular products may offer novel therapies to treat inflammatory diseases in humans.

What outputs do you think you will see at the end of this project?

Our primary outputs will be publications so that knowledge will be in the public realm. In addition, there may be commercial products based on, or using, the scientific results for therapeutic immunomodulators and/or new vaccine targets.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The impact of helminth modulators is likely to be high, in the longer term; we cannot predict which precise products will prove to be the most effective in treatment of inflammatory disorders, but the project is mining a rich vein of previously unexplored molecules. The impact on future vaccine development is also likely to be high, in the longer term, both through conceptually identifying the best strategy, and in taking specific vaccine antigens forward for clinical testing. In terms of vaccines, it is also important to factor in the impact on animal health, if we can prevent helminth infection in livestock across the UK and globally.

How will you maximise the outputs of your work?

We will maximise output not only through the conventional channels (formal publications, conference presentations etc), but also through new forms of communication (BioRxiv, the laboratory website and Twitter streams), plus new forums for recording robust but unsuccessful studies such as Wellcome Open Research.

Species and numbers of animals expected to be used

- Mice: 29750
- Rats: 1000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Two types of procedures will take place. In the simplest, we will infect mice and rats with parasites so that we can grow the parasites and collect molecular products from them.

These animals will be injected, or given parasites by mouth, just once. In more complex work, we will also induce in mice diseases corresponding to human disorders of allergy, multiple sclerosis and inflammatory bowel disease, which involves administering allergens, self-proteins and noxious substances to the airways by inhalation, the peritoneal cavity by injection, and the intestinal tract by mouth or rectal administration. Then test substances will be given to the mice by injection, by mouth, or by implanting miniature pumps that continuously release the substance. Typical experiments will last 1-2 weeks and the total number of animal used over 5 years is likely to be 10,000.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Most animals show no untoward effects of the parasite infection itself in the doses and time scales we use. However, to test the effects on inflammatory diseases, some animals will develop stressful signs including weight loss, respiratory or intestinal discomfort, diarrhoea or in very few cases, limb paralysis. These effects may last for up to 2 weeks, although in severe cases (such as limb paralysis) the animal would be culled humanely.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Sub-threshold - 40% of mice, 80% of rats.
Mild - 40% of mice, 20% of rats.
Moderate - 15% of mice
Severe - 5% of mice

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 13 November 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The parasites we study, the helminth worms, can only complete their life cycle in living animals, in our case mice and rats. The diseases we study are the incurable inflammatory

disease of humans (asthma, inflammatory bowel disease and multiple sclerosis) each of which is a complex combination of factors only found in the live mammalian animal.

What was your strategy for searching for non-animal alternatives?

Laboratory cultivation of parasites without need to infect live animals; laboratory production of antibody- like reagents from bacteriophage; use of cell lines and tissue explants.

Why were they not suitable?

Currently, it is not possible to cultivate the parasites to complete their life cycle entirely in the laboratory, although this may become possible in the future and we will continue to monitor progress in this area. In vitro techniques for antibody generation cannot reliably produce high affinity reagents due to the lack of mutated affinity matured sequences; cell lines and tissue explants do not incorporate the complex web of innate, adaptive and stromal cell populations which migrate and differentially expand in vivo.

A retrospective assessment of replacement will be due by 13 November 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

I referred to the annual usage of my research group over the past 10 years maintained at a similar level of staff members, having considered where we could reduce the number of animals used.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

At every step of the Protocols we have considered whether overall numbers can be reduced, refining for example techniques to minimise variation (and therefore group sizes) and taking advantage of recent innovations (for example single cell RNA sequencing) which greatly expand the data that can be recovered from individual animals, again obviating large group sizes. A further very significant development has been the method for growing laboratory cultures of intestinal cells which form "mini- guts" of differentiated, functional tissues reproducing the architecture of the intestinal tract, that allow us to test the effects of parasites and parasite products on intestinal epithelium, avoiding the use of procedures on live animals.

What other measures apart from good experimental design will you use to minimise numbers?

Our laboratory strives to make most efficient use of the animals available, synchronising workplans so that, for example, from a single donor mouse, some tissues (eg the spleen) are harvested as a source of lymphocytes (white cells) of the immune system, while intestinal stem cells are also recovered to form the cultures of "mini-guts" that grow to reproduce the architecture of the intestinal tract in laboratory- based experiments. Work to produce infective parasites is also organised to maximise efficiency and minimise animal numbers, with each round of infection serving to provide parasites for multiple procedures within the Project.

A retrospective assessment of reduction will be due by 13 November 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

The animal models we use for parasite infection cause minimal suffering or distress to the rodents as they are naturally well-adapted to their host. The models of inflammatory disease, however, do inescapably cause a degree of suffering as they are designed to replicate severe human conditions. Our approach is to select models (a) which are very well established and well characterised with respect to the course of disease; and (b) on this basis select a window of time for analysis which minimises any suffering the animals may experience.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The animal models have all been developed in laboratory mice, and no alternative species is known. The models take up to 6 weeks to develop and hence anaesthesia is not appropriate.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

We will continue to discuss within our regular laboratory meetings, and at every opportunity with our veterinary colleagues and NTCO. An early priority will be to test

smaller gauge needles for subcutaneous injection of parasites, to establish the optimum for animal welfare that does not damage the parasite larvae through shear forces within the needle. More broadly, we will encourage our research staff to attend courses such as the FRAME training school in experimental design.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Our principal guidance will be through the ARRIVE (Animal Research Reporting of In Vivo Experiments) guidelines available through the NC3Rs website. In addition, our Animal Welfare and Ethical Review Board (AWERB) also produces specific guidelines (eg most recently on single use of needles, also blood sampling procedures) that further refine and ensure best practice.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Our REDACTED has a very active system for disseminating information about best practice and new approaches for the 3Rs, including organising an annual 3Rs Symposium, and we are in frequent dialogue with our veterinary colleagues and our Named Training and Competency Officer (NTCO), about specific steps in the procedures we employ.

Explain the choice of species and the related life stages

Rodents provide natural hosts for parasites of the intestinal tract which are related to those infecting some 2 billion people in tropical countries; we will study the immune response of adult mice in order to develop new vaccines, and to understand how the parasites can switch off host immunity in a manner that would be useful for new anti-inflammatory therapies.

A retrospective assessment of refinement will be due by 13 November 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

30. Molecular basis of Gram-negative bacterial infection

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The global aim of this project is to study the ways in which bacteria which cause disease (bacterial pathogens) interact with the human body. In particular, we focus on infections caused by gut and lung bacterial pathogens. We study the mechanisms by which these pathogens cause disease, the impact of antimicrobial resistance (when bacteria become unresponsive to antibiotic treatment) and aim to develop novel treatment and/or prevention strategies.

A retrospective assessment of these aims will be due by 23 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Enteric (gut) infections remain a major public health issue, mainly in low and middle income countries. In particular, infections with pathogenic Escherichia coli are responsible for disease and death in young children. Currently there is no vaccine available for pathogenic E.coli infections and, as treatment with antibiotics increases the risk of secondary complications, treatment is limited to supportive treatments (e.g. oral rehydration).

On the other end of the spectrum, Klebsiella pneumoniae is a hospital-acquired infection that mainly affects unwell, mostly elderly patients in intensive care units. Lung infection with K. pneumoniae can lead to subsequent sepsis (multi-organ failure), which is often fatal. Importantly, due to the rapid spread of antimicrobial resistance, treatment of K. pneumoniae infections is becoming increasingly challenging.

Accordingly, it is essential that we increase our knowledge and understanding of the relationship between the pathogens and the host in order to combat these life threatening infections. Increased knowledge will aid the development of better treatments and vaccines and also the development of therapies involving bacteria which normally live in the healthy gut, which help to prevent infection. Such organisms are known as the gut microflora.

What outputs do you think you will see at the end of this project?

The outputs from this project will include modelling of serious gut and lung infections in humans. Basic science such as this is necessary to make advancements in clinical medicine. The overall aims of this project are to further our understanding of how these diseases develop and spread, and to improve global health.

Specifically, in the gut, our work will highlight the underlying factors that determine whether individuals develop mild or severe symptoms in response to bacterial infection. This will include understanding the role the immune system (the body's defence system against infection) and the normal gut microflora play during infection. Moreover, we aim to develop and evaluate novel vaccination strategies.

In the lung model the outputs of this project will include new understanding of the changes which occur during life-threatening sepsis. Further, many clinically-relevant Klebsiella pneumoniae are resistant to first-line antibiotic treatments. These infections are often mistreated in the clinic. We will investigate the effect of antibiotic mistreatment on the host and the pathogen.

These are emerging disciplines that will inform both scientists and clinicians, and the data will be disseminated via publications. We aim to enable public access to our work by paying for open access where possible. We disseminate our findings via the general press to inform the public of our work.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

In the short term (5 years), the project will contribute to basic scientific knowledge and set new parameters for studying bacterial infection. In the long term this knowledge will contribute to the development of more effective treatments for gut infection. Our novel

vaccination strategy will benefit those interested in disease prevention via vaccination, which is an essential strategy in the face of increasing antimicrobial resistance.

Understanding the impact of antibiotic mistreatment will enable us to model a very real world situation, where antibiotic resistance has rendered many of our previous therapies ineffective. In the long term, the development of a robust model of Klebsiella lung infection, which produces all the physical characteristics of disease, will benefit those interested in developing novel treatment strategies. We aim to use new technology to stop experiments at earlier, less severe time points in keeping with the RSPCA's recent publication of a 'road map' toward ending severe suffering of animals used in research and testing.

How will you maximise the outputs of your work?

We routinely publish our work in leading scientific journals and present our findings in national and international conferences. We have track record of involving animal technicians, and welfare staff (e.g. NACWOs, NTCOs and NVSs) in our work and results. We tend to include unsuccessful approaches together with reporting successful ones to inform the community and reduce duplication of failed approaches in animals (within our and at other institutions). We collaborate with the global infection research community using new techniques that provide rich data sets enabling fewer animal experiments to be required, yet providing more information.

Species and numbers of animals expected to be used

- Mice: 14250

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Mice will be infected with an enteric (gut) or pulmonary (lung) bacteria by oral gavage (delivery of bacteria via a feeding tube to the stomach) or intratracheal inoculation (delivery of bacteria via a tube into the lungs). In some cases this will result in clinical symptoms of infection including weight loss and illness.

Compounds such as drugs or antibiotics are delivered by either oral gavage, injection or via the anus. This is to study how disruption to normal bodily function affects the symptoms developed as a result of infection. We may collect blood from mice and/or image the mice during the course of infection to monitor infection progression. Where required for the above procedures, anaesthesia is given either via injection or inhalation.

The number of regulated procedures is dependent on the protocol used. Aside from symptoms developed as a result of infection, most procedures used in this project cause no more than transient discomfort and no lasting harm to the animals.

Experiments concerning lung infection are short (around 72 hours).

Gut infection experiments, depending on the type of mouse and pathogen, are longer and can last from 4 days to >3 months. In scenarios where mice develop symptoms of disease, these experiments will typically last less than 3 weeks.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Gut infection in non-susceptible mouse breeds (mice which spontaneously clear infection) only results in short-lived, mild, symptoms of disease and the mice spontaneously clear the infection after 20-30 days. Gut infection in susceptible mouse breeds (mice which, without intervention, would succumb to infection) results in gut inflammation (colitis), diarrhoea, weight loss and disease symptoms including reduced ability to move, hair standing on end, and development of a hunched gait (bent walking posture). However, animals will be killed (to prevent excessive suffering) when weight loss exceeds 20%. Additionally, we will use an agreed scoring method to ensure that animals will not suffer moderate symptoms for more than 48 hours.

Infection with Klebsiella will not cause pain, but these infections do induce sepsis and multi-organ failure (septic shock). Infections will typically not last longer than 72 hours, except when appropriate antibiotics are administered to mice, curing the infection.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Protocol 1 (gut infection) is a moderate severity protocol. The majority of mice (85%) are expected to experience only mild severity in which they do not show visible signs of infection. Mice susceptible to infection will sometimes be allowed to develop some symptoms (reduction in mobility, hunched posture, raised hair) and 20% weight loss. This (moderate severity) is expected to occur in 15% of the mice.

Protocol 2 (vaccine development) is a moderate severity protocol. The majority of mice (70%) are expected to experience a mild severity in which they do not show visible signs of infection. Mice susceptible to infection will sometimes be allowed to develop some disease symptoms (reduction in mobility, hunched posture, raised hair) and 20% weight loss. This (moderate severity) is expected to occur in 30% of the mice.

Protocol 3 and 5 (lung infection) are severe in which mice develop multi-organ failure due to infection and septic shock. This is applicable to all infected animals, with 90-100% reaching this severity end- point.

Mice under protocol 4 (breeding) are not expected to develop any symptoms as the resulting genetic make-ups are not predicted to have a harmful effect (mild severity).

What will happen to the animals at the end of the study?

- Used in other projects

A retrospective assessment of these predicted harms will be due by 23 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We study the way in which harmful bacteria (pathogens) infect the lung and gut and cause disease symptoms. Initially we investigate these bacteria in non-animal models as much possible. However the true mechanisms of infection can only be revealed using relevant animal models as we find that non- animal infection data does not reflect what happens in a complex organism (e.g. mouse or human). We aim to model the ways in which bacteria cause disease in the context of the gut flora, immune responses, metabolism and diet. Moreover, while infection of cells in the laboratory is studied in hours, infection in animals/humans can last from a few days to many weeks, with a delayed development of disease symptoms after the initial infection.

The use of animals is an unavoidable consequence of studying the complex processes involved in models of human infection. Furthermore, there are no non-animal models of the complex immune system, therefore testing vaccine candidates can only be done using animal models.

What was your strategy for searching for non-animal alternatives?

Where possible we use non-animal alternatives, e.g. cells and tissue grown in the laboratory, for our work.

Why were they not suitable?

Our aim is to model infection in humans, which requires investigating pathogen/host/microorganism (gut flora) interactions as a whole. Moreover, the outcome of infection is dependent on external factors, e.g. diet, that is not reproducible in the laboratory. Accordingly, whilst we investigate some specific questions in laboratory-based systems, there are no alternatives to animals as a complete model of human disease, and therefore development of treatment strategies.

A retrospective assessment of replacement will be due by 23 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of experiments is based on previous experience and the number of mice is based on statistical calculations which determine the minimum number of animals needed in order to provide meaningful results.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Several different bacteria are routinely tested in the same experiment so that control mice (i.e. mice that have not been infected, for comparison with those that have) are shared, thereby reducing the number of animals (3Rs principles). All experiments are repeated to achieve scientific validation and to ensure reproducibility so that the results are trustworthy.

Each mouse is marked and analysed individually. Each group of mice is assigned an experimental identification randomly and, where appropriate, the analysis is done blindly (to prevent bias). Statistical analysis has been validated by external consultation (Institution's statistical advisory service) and vigorous peer review in high quality scientific journals. By using statistical software we are able to calculate the number of mice required for each experiment (power calculations) to enable theory-driven research whilst utilising the minimum number of animals.

End-points are traditionally time-defined in scientific experiments but this does not account for the specific biological differences between animals, so we have invested in a novel system to monitor mice, similar to those systems found in hospitals and are working towards being able to apply this to other infection models. This will enable us to collect more data per animal to reduce the number of animals required in our research.

What other measures apart from good experimental design will you use to minimise numbers?

We use hi-tech animal imaging techniques to reduce the number of mice used for testing and adhere closely to the 3Rs principles. Before imaging techniques were refined, we had to humanely kill a group of mice for each time point of interest in order to collect data. However, imaging techniques have evolved so much that we can now follow the same group of mice over time and record data at various time points, without the need to kill them. This has allowed us to reduce the number of animals required to achieve the objectives.

A retrospective assessment of reduction will be due by 23 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

We use mice to model gut and lung infections in humans. For gut infection, bacteria are given to the animal by oral gavage. This procedure does not typically cause pain, distress or lasting harm.

For lung infection mice are anaesthetised before delivery of bacteria through a breathing tube. This procedure does not cause lasting harm, although mice will develop disease symptoms as a result of the infection.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

As we aim to model human infection, we need to use adult mice, which have a developed microbiota (similar to those of humans). As we follow infection and symptoms over time, we have to use live animals.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

On a day to day basis we visually monitor the animals. When required, we also use a numerical scoring system based on visual symptoms and weigh the animals. Non-invasive measuring of heart and breathing rate will allow us to define precise, more humane endpoints and monitor animals in real time and therefore reduce harm.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We routinely follow the NC3Rs ARRIVE guidelines.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We routinely follow the literature (both scientific and industrial/commercial) and conferences for new developments and technologies. We have evidence for this, as we have recently bought and implemented the MouseOX technology in our facility, which allows continuous monitoring of the health status of mice throughout an experiment.

Explain the choice of species and the related life stages

We use mice because there is natural and specific mouse pathogen (*Citrobacter rodentium*) which 20 years of research by us and others has been established as a robust and accurate model for human gut infection with pathogenic *E.coli. Klebsiella pneumoniae* causes an infection cycle in mice (from pneumonia to sepsis), which mimics its infection outcome in humans.

As we study infection in the context of the natural gut flora (microbiota) and monitor immune responses, we need to use adult mice which have developed a mature microbiota and have a mature immune system.

A retrospective assessment of refinement will be due by 23 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

31. Mouse models of lung cancer progression & therapy

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Our aim is to improve the understanding of lung cancer initiation, progression and disease spread (metastasis). Ultimately, by the use of our mouse models, we aim to develop new treatment strategies which can be applied to the clinic.

Retrospective assessment

Published: 22 February 2022

Is there a plan for this work to continue under another licence?

No

Did the project achieve its aims and if not, why not?

The project aims have been partially met as the Licence was terminated within 15 months of being granted because the desired objectives were achieved, and the research group

was closed down. The research has contributed to understanding of both how lung cancer forms and spreads by looking at how specific genes are expressed.

These models have been included in peer reviewed publications and data have been presented at internal and external scientific meetings thus sharing our findings with the scientific community. Additionally, we have published on the development of several mouse models of lung cancer in peer reviewed journals. These results have helped explain at how some lung tumours form in the first place and hinted at ways some tumours can be treated. Immediate beneficiaries from this research include both academic labs by providing insight into understanding how lung tumours form. Additionally, we have identified potential drug targets as well biomarkers for diagnosis and prognosis that pharmaceutical companies may wish to pursue.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Despite years of research, the prognosis for patients with lung cancer remains dismal. The most frequent type, non-small cell lung cancer (NSCLC), accounts for 85% of all lung cancer cases and shows an overall five-year survival of 15%. The long-term survival for patients with advanced high-grade lung cancer has been limited by the frequent occurrence of resistance to chemotherapeutic drugs often established by the onset of parallel compensatory mechanisms (Jemal, A., et al. Cancer statistics, 2009). In order to identify novel genes involved in chemoresistance as well in lung cancer progression that could be targeted therapeutically, mouse studies are fundamental. In vitro model systems have contributed to our knowledge of cell migration and invasion and allowed dissection of the role of individual genes in these processes. However, full understanding of these processes can only be achieved using in vivo models.

What outputs do you think you will see at the end of this project?

This project not only will contribute to understanding of the mechanisms, involving non-coding RNAs, of solid tumour initiation, progression and metastasis but will also help with the development of new miRNA / lncRNAs agents for use as a therapy. The models we shall use will elucidate these underlying mechanisms in mice and allow us to test the efficacy of targeted and conventional therapeutic agents and ultimately in surrogates for individual patients.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Immediate beneficiaries from this research will be other academics across clinical and scientific fields, working in the field of lung cancer and non-coding RNAs. They will benefit from the potential outcome of this study that will lead to novel biological hypotheses. Potential other beneficiaries include pharmaceutical companies that actively develop novel medicines for cancer therapy, as well biomarkers for diagnosis and prognosis. Data from this study will enable the identification of molecules that could be exploited by pharmaceutical companies as novel therapeutic targets. Potential beneficiaries could be cancer patients, especially those with advanced disease, for whom at the moment a cure

is not available. Lung cancer patients will be the direct beneficiaries of these discoveries in the long term for the potential development of treatments that will result in improved survival and/or enhanced quality of life.

How will you maximise the outputs of your work?

We shall publish our work in peer-reviewed journals, present the data at internal and external scientific meetings thus sharing our findings with the scientific community.

Species and numbers of animals expected to be used

- Mice: 5600

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Some mice (bred under other Project Licences) harbour genetic modifications that pre-dispose them to the development of lung cancers when exposed to an appropriate agent such as Cre recombinase or a carcinogen. Furthermore, some genetic modifications allow us to track specific cells within the tumour (such as T cells expressing luciferase). In other mice, which are bred specifically to tolerate human tissue, tumours will be implanted under the skin of the mouse for ease of monitoring or into the lung or into the blood stream to study cancer spread (metastasis). Mouse cancer cell lines can be transplanted into mice sharing the same genetic background (so called syngeneic mice) without rejection. Tumour growth is not associated with pain during the period in which we conduct our observations. Tumour growth will be monitored regularly by either use of callipers for superficial tumours, or by imaging methods such as CT for internal tumours. For some procedures that involve surgery under general anaesthesia, such as implanting human tumour fragments or removing a primary tumour in order for secondary tumour to grow, we will administer pain killers (including contained in jelly) and monitor the mice closely during recovery.

Some mice may have either potential novel therapeutic agents or genetic therapy molecules, existing clinical agents or a placebo administered by a variety of routes, but usually either by mouth, or by injection either under the skin or into the abdomen to study the effects on tumour growth and / or tumour composition. The mice will also have blood samples taken either from the tail vein or by sampling from a heart chamber under anaesthesia (in which case the animal does regain consciousness before humane termination). Occasionally mice may be administered an organ preservative whilst under non- recovery anaesthesia to allow us to undertake histology investigations on slices of selected organs.

Mice may be studied for up to 90 days after a period of therapeutic agent treatment for tumour growth. Fast growing tumours will be monitored daily.

Mice will be group housed in ventilated cages which have their environment enhanced with items such as tunnels, houses, nesting material and gnawing blocks.

At the end of any protocol mice will be humanely killed.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

The impact of the gene modifications are not expected to cause any adverse effects per se other than, in the case of the tumourigenic mutations, promoting the propensity to produce tumours. It is possible that the tumour growth might affect normal physiological functions (such as eating, locomotion or breathing) however, mice will be observed daily and any side effect that cannot be managed satisfactorily will be killed humanely. Injections would only cause very transient pain.

After surgical procedures we will monitor mice for signs of pain and administer effective pain relief for as long as it is required.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The vast majority of mice are only expected to experience the mildest clinical symptoms due to tumour growth before they are humanely sacrificed. Additionally, some mice will experience the discomfort of repeated (daily) injections of therapeutic agents or oral delivery with a specialist tube. We will am to utilise the least stressful route of administration wherever possible.

A minority of mice will undergo surgery and these will be anaesthetised for the operation and receive pain killer post-operatively until pain subsides. Some mice will also have repeated anaesthesia for the purposes of imaging the internal tumours. Whilst loss of consciousness may be distressing this is not painful.
Finally, a small number of mice will be used for determination of metastatic potential of cells. As this will involve intra-cardiac injection of cancer cells we do not know where they may be distributed, and therefore know the location of metastatic tumour growth. There is a small risk that there could be Severe endpoints in these mice but will be mitigated by regular monitoring. After characterisation of the clinical signs it may be possible to reduce the severity band in future use.

What will happen to the animals at the end of the study?

- Killed

Retrospective assessment

Published: 22 February 2022

What harms were caused to the animals, how severe were those harms and how many animals were affected?

A total of 121 mice were used. Tumour growth was never allowed to exceed the strict study parameters and all mice were culled humanely at the designated time-points, or when showing signs of breathing abnormalities, as per the study protocol (maximum of 20 weeks from initial treatments).

Body weight was measured once a week and mice were observed for signs of illness or distress as rapid weight loss (>20%) and laboured respiration. At the designated time-points, or when showing signs of breathing abnormalities, mice were sacrificed, and suitable samples were taken to meet our research goals.

Most mice experienced clinical symptoms such as discomfort and skin irritation or shortness of breath due to tumour growth before they are humanely killed. Additionally, some mice experienced the discomfort of repeated daily injections or oral delivery (via specialist tube) of therapeutic agents.

Tumour growth was not associated with pain during the time-course of the studies. Tumour growth was monitored regularly by either use of callipers (for tumours growing under the skin) or by imaging methods (for tumours growing internally). For some procedures that involved surgery under general anaesthesia, such as implanting human tumour fragments or to remove a tumour, we administered pain killers and monitored the mice closely during recovery. The mice also had blood samples taken under anaesthesia to help monitor the tumour.

Group allocation and monitoring were performed by unbiased colleagues of the Establishment animal husbandry team who were not aware of which mice received which treatments. Sample analyses were also performed by staff who were unaware of whether treatment was the trial or the standard one, to prevent bias. Established statistical techniques were used to ensure that the study was adequately designed to answer the research question.

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

While valuable studies of human cancer are performed using tumour material and cell lines derived from both mice and human samples, the mechanistic understanding of cancer pathogenesis requires use of living animals. In particular, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding host and their behaviour is governed by multiple signals originating from both their immediate neighbours and from distant tissues.

Transgenic mouse models have been engineered to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. This cannot be replaced by in vitro studies or indeed even in different in-vivo models such as zebrafish or insects which remain far less complex than their murine counterparts.

What was your strategy for searching for non-animal alternatives?

We will use a variety of in vitro approaches to investigate how manipulation of nucleic acid based targets alter cell behaviour in cultured cancer cells (lung,) prior to undertaking in vivo studies. Methods to be utilized include cell biology techniques to measure cell proliferation, survival, migration, invasion, etc., biochemical and molecular biology techniques such as western blotting, enzymatic assays, proteomics, RT-PCR, etc. to study protein function. In addition, we use molecular pathology (e.g. immunohistochemistry) to substantiate findings from our in vitro models in human tumour samples.

Why were they not suitable?

The study of cells in culture (in vitro) provides us with clues on the mechanisms of cellular processes in a simple and valuable context, which allows the establishment of hypotheses regarding the function of cells in a living animal. However, these systems do not recapitulate the complex cellular interactions described above.

Retrospective assessment

Published: 22 February 2022

What, if any, non-animal alternatives were used or explored after the project started, how effective were they and are there any lessons worth sharing with others?

Use of molecular biology and cell culture were used to replace the need to use mice. Many mechanistic and functional studies were able to be undertaken in the lab by studying lung cancer cells grown in a dish in the lab. These studies helped us understand how different mutations affected the cancer cells and how we might use this information for the treatment or diagnosis of patients.

Additional studies used human tissue sections to investigate the expression of biomarkers in patient samples to substantiate our in vitro and in vivo models. Briefly, samples from both patients with and without lung cancer were obtained from the local Biobank under the Hospital Human Tissue License and written informed consent was obtained from all patients.

Wherever possible, computer modelling and patient databases were also exploited instead of the mouse models.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The overall aim will be to generate models whereby a measurable effect e.g. reduction in tumour volume or tumour incidence following manipulation of a gene of interest or treatment with a drug can be determined using a minimal number of animals.

Data available from the literature or from pilot studies are used to perform power analysis to determine an appropriate sample size for the definitive experiment. In general, we will use a sample size capable of detecting a 40% practical difference with 80% power and 95% confidence.

Based on past experience, group sizes of between 10 and 30 animals (dependent on the readout, fewer for transplanted tumours compared to spontaneous tumours in GM mice) per experimental group suffice. However for an experiment to be well controlled and meaningful, we may include more than one experimental group. For instance, in implantation experiments where we deplete a gene in a cell line using shRNA, we will use two independent shRNAs targeting the gene as well as a control shRNA. Moreover, we would typically examine more than one model cell line. Likewise, we may use several doses of a drug, or several different drugs or drug combinations to test a theory. Considering power, the number of experimental groups, and the number of genes and drug targets we are interested in, we have then estimated the total number of mice to be used over the licence lifetime.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Use of in vitro methods limits the number of animals required for the in vivo investigation stage.

For transgenic models, efficient breeding strategy will minimise the number of mice used to obtain the desired genotype.

Experiments will be appropriately controlled and mice of the same age, genetic background and source used to reduce the variability of results and to produce highly consistent data. Wherever possible and appropriate, a single group of animals will serve as a control for duplicate experimental group. The proposed experimental designs and methods of analysis of the results will follow statistical guidelines and involve discussion with our bioinformatician scientist to provide sufficiently powered studies, minimizing the number of animals used in each experiment. The design of individual experiments will generally involve factorial designs, which maximise the information obtained from the minimum resource.

We will be conducting and recording our experiments to be able to publish our results following the ARRIVE guidelines [https://www.nc3rs.org.uk/arrive-guidelines] and will use randomisation, blinding etc. where appropriate so as to minimise biases. Furthermore, additional resources may be used to aid experimental design such as the NC3Rs experimental design assistant tool

(https://www.nc3rs.org.uk/experimental-design-assistant-eda).

What other measures apart from good experimental design will you use to minimise numbers?

Pilot studies will be performed if applicable and, after analysis of the results, group sizes for subsequent experiments will be determined based upon these data. As far as possible, multiple parameters will be evaluated in a single mouse. Live imaging of the same animal at multiple time points also greatly reduces the numbers required.

Retrospective assessment

Published: 22 February 2022

How did you minimise the number of animals used on your project and is there anything others can learn from your experience?

Use of in vitro methods limited the number of animals required for the in vivo investigation stage.

Experiments have been appropriately controlled and mice of the same age, genetic background and source were used to reduce the variability of results and to produce highly consistent data. Wherever possible and appropriate, a single group of animals served as a control for duplicate experimental group.

The experimental designs and methods of analysis of the results followed statistical guidelines and involved discussion with our bioinformatician scientist to provide sufficiently powered studies, minimizing the number of animals used in each experiment.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Mouse models that we are currently using faithfully recapitulate the human disease. Moreover, the mouse genome shares 98% homology with human genome.

We constantly work to improve husbandry and procedures which minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. We ensure to provide the appropriate anaesthetic and analgesic regimes as well as appropriate humane methods of culling within animal facility. We ensure no visualisation of procedures in other mice and transport arrangements between facilities in appropriate carrying boxes. We will use non-invasive imaging procedures to follow lung cancer and metastatic tumours. When the scientific endpoint has been reached the animals will be killed before any humane end-point is reached.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Less sentient animals do not have lungs. Mouse is far more similar to humans than other animals having lungs e.g. birds or reptiles and this is critical both for using reagents like drugs developed for human targets and for translating findings to the clinic. Cancers develop over many weeks to months, so use of terminally anaesthetised animals or immature animals is not practicable. Also immature mice lack a functional immune system which is desirable in cancer research.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Assessment of the size of superficial tumours would usually be by callipers (usually of two diameters at right angles). The total tumour burden should not normally exceed 1500 mm^3 as calculated by the formula: tumour volume = ½(length x $width^2$). Sub-cutaneous tumours will be monitored at appropriate intervals (usually once a week and measured by callipers). If a tumour reaches approximately 2/3 of the maximum permissible volume, it will be measured more frequently.

Growth of internal tumours will be monitored by a combination of palpation and, where possible, non- invasive imaging. Wherever relevant, animals will be provided with analgesia as detailed in the relevant Protocol to control adverse symptoms associated with any surgical treatment.

Provision of nursing support, such as mash, analgaesic gels.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Relevant published literature will be used as template for experimental design and decision making (Workman et al., 2010. Guidelines for the welfare and use of animals in cancer research. BJC, 102, 1555-1577).

We will follow guidelines of good practice [Morton et al., Lab Animals, 35(1): 1-41 (2001); Workman P, el al. British Journal of Cancer, 102:1555-77 (2010)] administration of substances will be undertaken using a combination of volumes, routes and frequencies that themselves will result in no more than transient discomfort and no lasting harm.

Guidelines for Body condition score. [Ullman-Cullere, Lab Anim Sci. 1999 Jun;49(3):319-23]

How will you ensure you continue to use the most refined methods during the lifetime of this project?

By reading 3Rs literature and participating in 3Rs workshops locally and nationally. Through discussing refinements with our NACWO, NVS and HO inspector. I am also a member of the AWERB at the REDACTED.

Explain the choice of species and the related life stages

Mice are more comparable to humans than less sentient model systems (fish, invertebrates) in pathophysiology and show higher levels of conservation in nucleotide and amino acid sequences. This is important as we intend to use reagents such as small molecule inhibitors and antibodies that have been developed to target human proteins.

Moreover, non-protected species and less sentient species do not have lungs, so we would be unable to use them for animal models of lung cancer involving the injection of lung cancer cells in the organ where they are found in man. Embryonic stages would not provide us with a sufficient window to follow tumour development and besides it is not

feasible to perform the desired interventions in embryos (such as inhalation of Cre-expressing virus). Therefore adult mice are to be used.

Retrospective assessment

Published: 22 February 2022

With the knowledge you have now, could the choice of animals or models used have been improved at all? How did you minimise harm to animals during the project?

The mouse models that we used recapitulate the human disease. Moreover, the mouse genome shares 98% homology with human genome.

We worked to improve husbandry and procedures which minimise actual or potential pain, suffering, distress, or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable.

We ensured that appropriate anaesthetic and analgesic regimes were used as well as appropriate and Home Office accepted humane methods of culling within animal facility.

We used non-invasive imaging procedures to follow lung cancer and metastatic tumours. When the scientific endpoint was reached the animals were killed before the mice became ill.

Guidelines we followed:

Workman et al., 2010. Guidelines for the welfare and use of animals in cancer research. BJC, 102, 1555-1577.

Morton et al. 2001, Lab Animals, 35(1): 1-41.

Guidelines for Body condition score. [Ullman-Cullere, Lab Anim Sci. 1999 Jun;49(3):319-23]

By reading 3Rs literature and participating in 3Rs workshops locally and nationally. Through discussing refinement.

32. Novel therapies for childhood blood cell cancer

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

Contains severe procedures

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The purpose of this project is to develop new treatments for blood cell cancer and other blood diseases in children. Research is urgently needed to find new cures for children whose disease cannot be treated with current chemotherapy.

A retrospective assessment of these aims will be due by 13 July 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The potential benefits of this work will be the development of specific treatments for childhood blood diseases, such as leukaemia, which will be better at treating the disease and will have fewer toxic side effects.

What outputs do you think you will see at the end of this project?

Blood cell cancer still accounts for just under a third of all childhood cancer deaths. New therapies are urgently required for children who cannot be successfully treated using current methods. This project aims to identify new therapies and to test how effective they are in blood cancer experimental models. Our aims in this project are to identify and test how effective different drugs are at killing cancerous blood cells. We will use drugs that we have shown are acting specifically on cancer genes. This research will generate a substantial amount of high-quality data that will be submitted for publication in international scientific journals, and communicated to scientific and clinical colleagues, in scientific conferences, and to members of the public, in outreach events. We envisage this happening within the time-frame of this PPL (5 years). Furthermore, our research involves the use of a number of laboratory techniques which we use for identifying new treatments. A likely output is therefore the development of new specialised experimental techniques that can be used in the laboratory.

Who or what will benefit from these outputs, and how?

Communication of the research, through publication and presentation at scientific meetings, will benefit scientists and clinicians involved in leukaemia and cancer research. This will lead to greater understanding of the disease process in children and fostering international collaboration in efforts to develop new treatments for children who do not survive current treatment. In addition to these shortterm benefits, in the long-term we envisage that this research will have a direct clinical impact within 510 years, resulting in improved therapy for children with poor prognosis blood cancer and decreasing the burden of conventional chemotherapy in many others. By demonstrating the anti-blood cancer cell properties of the drugs, we will be able to develop clinical trials for their use in patients. If successful, this will result in the cure of children who fail blood cancer therapy at present and diminish the longterm side-effects of chemotherapy-related toxicity. Furthermore, some of the therapies may well benefit adult blood cancer patients, whose disease is similar to those studied in this project. Adoption of novel treatments in clinical trials (5-10 years) and eventually in clinical practice (10-20 years) will benefit patients with blood cancer that is difficult to treat. The development of new specialised laboratory techniques are likely to benefit scientists and clinicians involved in blood cancer research, within the time-frame of this project (5-10 years).

How will you look to maximise the outputs of this work?

We will ensure that the knowledge gained from this project is shared with the scientific and clinical community by publicising it as publications in international scientific journals and presentations at national and international scientific meetings and scientific and clinical workshops. The latter will also be particularly important in sharing experience of negative

data, since this will inform future experiments and experiments on new treatments. In the event that negative data would potentially challenge current or future directions of research, we will aim to publish these in international scientific journals.

Species and numbers of animals expected to be used

- Mice: 5500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use mice in this project. Normal blood cell development and blood cancer has been studied in mice extensively and has been shown to be very similar to that in human beings. The information obtained by studying these biological processes in mice is therefore highly relevant to human blood development and disease, including blood cancer. Mice are required for certain experiments, because when these are performed in the laboratory, they do not reflect how cells behave inside the body. Mice can be used for these studies. For many experiments in this project we will use genetically altered (immunodeficient) mouse strains that can be used as hosts for the transplantation of human cells. This allows an even closer approximation to how normal blood development and blood cancer progression will be affected by future new treatments in patients.

Mice will be bred so that they are suitable for transplantation with normal or blood cancer cells, and exposed to new and existing treatments. Apart from breeding, our experiments will be limited to adult mice. Adult mice are the most appropriate stage for these experiments, which have been standardized previously in our laboratory.

Typically, what will be done to an animal used in your project?

Typically mice will be transplanted with mouse and human blood cancer cells or normal blood cells.

Normal blood development or cancer will be analysed by sampling peripheral blood or through using imaging techniques that are non-invasive. No mouse will be bled more frequently than 4 times in any 28 day period and no more than 10 times in total. For imaging analysis, mice will be anaesthetised for a maximum of 2 hours and imaged for no more than 30 minutes.

Mice may be treated with chemical substances and drugs to interfere with the development of blood cancer.

Mice will be kept for no more than 1 year following transplantation.
All animals will be killed by a Schedule 1 method at the end of the experiment.
In some cases, it may be necessary to house mice individually, where other mice in the cage have been sacrificed due to disease progression. This will be done in order to evaluate disease progression in all mice in a given group and/or evaluate the effectiveness of novel drugs. The duration of individual housing will be no longer than one year, and in most cases between 1-3 months.

What are the expected impacts and/or adverse effects for the animals during your project?

Some mice will show signs of clinical illness, resulting from development of blood cancer or the failure of normal blood cell development. Such clinical symptoms may be weight loss (up to 20%), abnormal coat, segmentation of vertebral column, readily palpable dorsal pelvic bones and subdued behaviour patterns. This will mostly be a result of blood cancer progression in transplantation recipients. Any animals showing one of these symptoms will be monitored daily and if there is no improvement after one week, they will either be sacrificed or the named veterinary surgeon will be consulted. Any animals showing more than one of these symptoms will be monitored daily and if there are no signs of improvement after two days, they will either be sacrificed by a schedule 1 method or the named veterinary surgeon will be consulted. In a small number of cases, some mice may die without showing any prior clinical adverse symptoms, despite close monitoring. Individual housing may result in stress and abnormal behaviour. This will be minimized by environmental enrichment and limiting the duration of individual housing to that essential for the experiment. Where possible, for example female mice, a companion untreated mouse may be added to the cage for company.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

We expect that 10-40% of mice in this project to experience moderate clinical symptoms, and less than 2% to experience severe symptoms. We expect approximately 2-5% of mice will be individually housed at some point. The duration of such housing for most of these mice (>90%) mice will be 1-2 weeks, 1-3 months for <10%, and rarely for up to a year (<2%).

What will happen to animals at the end of this project?

- Used in other projects

A retrospective assessment of these predicted harms will be due by 13 July 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We need to use mouse models in this project in order to accurately examine the anti-blood cancer efficacy of interfering with particular cellular pathways in the body of an animal.

Furthermore, we require mice in order to determine whether the new treatments we are testing can eliminate blood cancer cells without killing normal blood cells.

Which non-animal alternatives did you consider for use in this project?

We plan to make use of extensive laboratory models to investigate which genes and pathways are essential for blood cancer cells to survive, but are not required for normal blood cells to develop. This will involve using molecular tools to target particular genes and drugs to block specific cellular pathways. These experiments will be done in the laboratory. We will make use of recently developed ways of growing blood cancer cells in the laboratory that will enable us to test different new ways of blocking the growth of blood cancer cells and killing them. Only those treatments that are successful in the laboratory will be taken further for testing in mice.

Why were they not suitable?

Although these laboratory experiments are important and complement research in mice, they are limited in how closely they can reproduce disease occurring in patients and are not sufficient by themselves to justify starting clinical trials in children diagnosed with blood cancer. In order to be sure that our experiments are likely to succeed in human patients, we need to study how blood cancer cells respond to new treatments we develop in an organism, in this case the transplanted mouse. This is because the treatment may be less effective in an organism, for example because the drug does not last enough in the blood, or because the bone marrow cells protect the blood cancer cells from the drug.

A retrospective assessment of replacement will be due by 13 July 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated the numbers of mice we will use in this project using mathematical calculations and experience from previous animal experiments. These numbers will be kept to a minimum while ensuring that the experiments are able to give us reliable information and results.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The number of mice used in this project will be kept to a minimum. Experiments in mice will only be performed if there is good evidence from laboratory experiments that the work

in mice is likely to succeed. For example, new treatments will only be tested in mice once they have been shown to be effective at killing blood cancer cells in the laboratory. Wherever possible, we will use new techniques to minimize the numbers of mice used. For example, we routinely use a method to analyse the extent of blood cancer growth in mice that is based upon using a special camera that can detect the cancer cells inside the mice. The use of this camera allows us to determine which new treatment is effective at blocking the growth of cancer cells without having to sacrifice the mouse, and this technique will therefore reduce the number of mice necessary to identify an effective new treatment.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The estimated numbers will be reviewed following completion of particular experiments examining how effective particular treatments are at blocking blood cancer growth. If the treatments are very effective, we will need fewer mice in future experiments using these treatments, and new mathematical calculations will be used to determine how many mice we can reduce in these. Pilot studies will be used to confirm that appropriate amounts of drugs are being used in the treatment of blood cancers. This will optimize experiments designed to test how effective these drugs are.

A retrospective assessment of reduction will be due by 13 July 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project will only use mice as the experimental animal. These are the most suitable animal to use based on a large amount of evidence from previous experiments by scientists all over the world and our current knowledge regarding blood development and blood diseases in mice, which are similar to those in human beings in many ways. Significantly, a recent study has shown that mice transplanted with human blood cancer cells respond in a similar way to treatments used in people suffering from the same types of blood cancer. We will use the most sensitive and least invasive method to examine disease progression in experimental mice wherever possible. This will reduce distress and suffering, as well as provide more accurate information and allow us to end these experiments at earlier time-points, reducing the suffering and distress experienced by the mice used.

Why can’t you use animals that are less sentient?

Normal blood cell development and blood cancer has been studied extensively in mice and mice represent the most accurate and least sentient species for this research. Adult mice and necessary to study these processes, because of the time-scales involved.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will examine and monitor mice regularly. Any mice showing any clinical signs of being unwell will be closely monitored by daily inspection. We will seek to use the most sensitive and least invasive method to examine disease progression. Any mice showing unacceptable signs of discomfort or disease will be humanely killed. We will keep up to date with new developments in animal experimentation in the UK and abroad that discover new ways of improving our experiments and minimising suffering of experimental animals.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The experiments will be designed, in accordance with ARRIVE guidelines, to detect differences between control and experimental groups in measurements of differences in blood cancer growth and normal blood cell development. For all experimental procedures, guidelines as published by Workman et al (Workman, P., et al., Guidelines for the welfare and use of animals in cancer research. British Journal of Cancer, 2010. 102: p. 1555-1577) and the LASA Good Practice Guidelines for Administration of Substances (October 1998) will be followed.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Attending external conferences and seminars on animal welfare and consulting the NC3Rs website - https://www.nc3rs.org.uk/

A retrospective assessment of refinement will be due by 13 July 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

33. Organ dysfunction and survivorship following acute illness

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	juvenile, adult
Rats	juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The overall objectives of the project are to (i) examine the response to disease processes encountered in critically ill patients, (ii) assess the impact of potential therapies and (iii) develop novel monitors/diagnostics.

A retrospective assessment of these aims will be due by 06 July 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Organ dysfunction following critical illness constitutes a continued major healthcare burden affecting both quality and duration of life. This work will assist in better understanding of the underlying biology of shock states, where the body cannot deliver enough oxygen to the tissues of vital organs, or the organs fail to use the oxygen appropriately. We place a particular emphasis on major body (metabolic, immune, circulatory, and hormonal) systems, their responses to specific insults, between-system interactions, and responses to therapeutic interventions. We will continue to explore similarities and differences in response between diverse acute illnesses, for example, haemorrhage, sepsis (a life-threatening condition that arises when the body's response to infection injures its own tissues), heart attack and stroke. These studies will also be used to evaluate novel diagnostics, monitoring technologies, biomarkers (chemical warning signs in the bloodstream), and novel therapies that may either offer protection or enhance recovery.

What outputs do you think you will see at the end of this project?

We propose to continue our current line of investigation into the effects of shock and sepsis in critical illness to better understand how the progression to organ failure occurs. We will also further our knowledge of disease processes by exploring common biological mechanisms between shock, sepsis and organ-specific medical conditions such as heart attack and stroke. Armed with this knowledge, we will attempt to repurpose existing drugs and develop new drugs that can potentially lead to protection, prevention or faster resolution of organ failure, and continue to develop new monitoring technologies and devices that can improve the care of patients in intensive care. We will present our research at national and international conferences and publish our work in high-impact journals. This aims to inform other clinicians and researchers of our progress, and often to enables a collaborative approach to achieving our common goals.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Immediate scientific benefits likely to be gained from this programme of work (1+ years)

This work will assist in better understanding of the underlying biology of shock states, with particular emphasis on circulatory, immune and metabolic abnormalities seen in these conditions. We will explore common mechanisms in diverse conditions such as shock, sepsis, heart attack and stroke. These studies will also prove highly useful in the evaluation of novel diagnostics, including new monitoring technologies and biomarkers (chemical warning signs in the blood stream), and novel therapies that may either offer protection in shock states, or enhance recovery.

Longer term benefits related to how human patients may eventually benefit (3+ years)

Better appreciation of the processes underlying shock and acute critical illness states, and assessment of the responses to different therapies, will facilitate the design of clinical trials and the development of therapies and monitoring technologies that can improve patient

outcomes. This could have significant ramifications for both improving quality and duration of life, and reducing the high financial cost of caring for these very sick patients.

How will you maximise the outputs of your work?

Over the last 20 years we have collaborated with >30 groups of diverse researchers from universities, biotechnology and pharmaceutical companies. This combined knowledge often led to scientific progress that neither us nor our collaborators could have achieved alone. We aim to continue this collaborative approach over the next 5 years with a view to better understanding disease processes and developing products that can improve patient outcomes. We inform others of our progress by presenting our work at national and international conferences, and writing up our results for publication in high impact journals. We will also aim to better inform the public and other researchers of our work by starting an online blog/twitter account. This could be very useful for disseminating knowledge of unsuccessful research (e.g. a novel therapy that did not work) that would otherwise be difficult to publish in high impact journals.

Species and numbers of animals expected to be used

Mice: 350
Rats: 7000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

In all of our protocols we have an optional pre-treatment phase where we may give drugs or alternative diets to see if they improve the response to a disease condition. Thereafter, we run three distinct protocols.

Short-term anaesthetised model. These experiments are performed over one day under terminal anaesthesia. This allows us to perform more complex surgical procedures than we could if the animal was to recover. This is important to be able to test specific organs to better understand how disease processes and responses to treatment(s) occur in different parts of the body, and how they interact with one another. We induce conditions similar to that encountered in an intensive care or emergency medicine setting, such as blood loss, heart attack and bacterial infection. The animals are euthanised at the end of the experiment with an anaesthetic overdose and do not wake up.

Long-term acute illness models (with or without metabolic monitoring). These are recovery experiments that we perform over 1-3 days, and under exceptional circumstances, up to 6 weeks. Typically, we perform a surgical procedure to insert special tubes into an artery and a vein. This allows us to be able to provide standard care that would be encountered on an intensive care unit, such as delivery of fluids and drugs, and blood pressure monitoring. These tubes are connected to a harness that, on recovery from anaesthesia, allows the animal unimpeded movement around its cage with free access to food, water, bedding and toys that enrich the environment. Sometimes we place the animals into

'metabolic' cages that allow us to measure their whole-body oxygen consumption. We typically induce a septic condition by administration of bacteria into the abdomen to model abdominal sepsis, a common condition in intensive care units. We frequently perform an echocardiogram after 6 hours (under brief anaesthesia) to assess the function of the heart, and this allows us to predict the severity of the disease course. We visually assess the animals at least 4 times a day and document their severity using our Clinical Scoring System. We monitor the animals more frequently in those that are predicted to be sicker. We sometimes give different drugs or fluids to assess their impact on the disease course. At pre-specified timepoints, usually 6 or 24 hours, the animals are re-anaesthetised. We then take blood and tissues from different organs for further study. The animals are euthanised at the end of the experiment with an anaesthetic overdose.

Survivorship model. These are recovery experiments performed over 14 days and exceptionally up to 7 weeks. Typically, a lower quantity of bacteria, fungus or inflammatory product is injected into the abdomen under anaesthesia to induce a less-severe disease course. The animals recover and are tested using methods to assess their survivorship, or quality of life. For example, this could include an assessment of their exercise capacity using a treadmill to see how well the heart and circulation can perform. It could also include tests to assess brain function such as interest in a new toy or exploratory behaviour in a new environment. At pre-specified timepoints, usually 14 days, the animals are re- anaesthetised. We then take blood and tissues from different organs for further study. The animals are euthanised at the end of the experiment with an anaesthetic overdose.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Adverse effects during the pre-treatment phases of experiments (e.g. drug administration) are not generally anticipated. Pain relief is given to animals recovering from operations. Animals are always observed until full recovery from anaesthesia, and regularly monitored throughout the day and early evening. Our considerable experience with these experiments reveals no clear evidence of surgically related infection or other complications. Following the administration of e.g. bacteria, animals may show characteristic ‘shock’ symptoms within a few hours. These are documented regularly (minimum 4 times daily) with clear end points offered by the Clinical Scoring System that we use. Any animal that is deemed to be suffering unduly is promptly culled. As noted above, we also use biological measurements (e.g. heart rate) to predict outcomes. Those predicted to be sicker are monitored more closely and additional support provided (e.g. mashed food, extra bedding, pain relief) as needed.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Our protocols have ‘moderate’ and ‘severe’ severity limits. The majority of experiments (>86%) will fall under the moderate category where severity limits are strictly imposed using our scoring system. A limited number of experiments (14%) will be performed under a severe severity limit although some of these will be controls (and are therefore unlikely to show a severe phenotype). The severe severity limit experiments are performed exclusively in rats for which we have developed a Clinical Scoring system to accurately

monitor their health status, and act upon this accordingly. In human patients, the effectiveness of a treatment for a critical illness is measured by improved survival. Although we generally aim to terminate our studies at pre-specified times, we feel there is an important place for a protocol to provide firm verification of benefit. At experiment end all animals are culled humanely.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 06 July 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The complexity of sepsis and other critical illnesses, involving multiple biological pathways (e.g. heart and circulation, immune and hormonal systems, and metabolism) means that we need to use whole animals to see the ‘big picture’. This work will both complement and enhance concurrent studies on patients where the inability to sample vital organs such as liver, gut and kidney means we need to use comparable animal models to further our understanding of these conditions. Furthermore, potential new therapies need to be assessed in whole animal models to make sure they are safe and that they work before testing them in patients.

What was your strategy for searching for non-animal alternatives?

Where possible, we use cell cultures to address specific biological questions. In the future, we will also consider technologies such as “organ-on-a-chip” but they are still in their relative infancy.

Why were they not suitable?

Although some other researchers claim to have translated organ functions onto chips, this technology remains in its infancy. As such, they cannot currently replicate the complexity of a whole-body system, how organs interact in health and disease, and how they respond to treatments.

A retrospective assessment of replacement will be due by 06 July 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Each group of animals that receives an insult such as an infection, we typically study 6-8 animals within that group. Our previous work indicates this is usually sufficient to show statistically significant differences compared to controls. The limited number of survival studies that we perform usually requires group sizes of 12-18 individuals. This is required to demonstrate statistically significant differences between groups in terms of time to survival or, more usually, numbers surviving to a fixed timepoint. We use power calculations for our survival studies, where we estimate the sample size we need using an online calculator.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

We match in terms of age, weight and sex to minimise variation in our studies. We also perform control studies on the same day to minimise environmental variation. Our controls are as closely matched to the study group as we can make them. For example, if a new therapy is being assessed, the control animals will receive the insult (e.g. infection), an equivalent standard-of-care protocol (e.g. fluid resuscitation), but not the treatment under study. Where possible we also aim to combine groups. For example, if we are assessing more than one therapy or intervention at the same time, we would use only one control group, thus reducing the total number of animals we would otherwise need to use. We have statisticians based in our lab who help us with our experimental design.

What other measures apart from good experimental design will you use to minimise numbers?

At the end of all of our experiments, we take blood and tissues so that we can gain more information and make maximum use of each animal studied. We often combine non-treated tissue or blood samples which can be shared out. When we do survival studies we may include an interim analysis to see if a treatment looks like it may be working. If the interim result shows no difference in the treatment, or if it appears harmful, we would terminate the study early to prevent further animals being unnecessarily used.

A retrospective assessment of reduction will be due by 06 July 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Though rodent models do not exactly mimic human pathophysiology, the same intrinsic pathways exist. In human critical illnesses, continual monitoring and support of multiple organs are necessary to improve survival. In order to study the biology of organ failure in animal models, it is likewise usually necessary to monitor the animals and their organ function frequently, and to support them. This results in models that are complex but more closely mimic a critically ill patient in an intensive care unit setting. Thus, the effects of novel therapies in our models are more likely to be relevant to the human condition.

The long-term awake animals do not appear to suffer unduly, either as a consequence of surgery and instrumentation (they are usually ambulatory and eating/drinking within 30 minutes of discontinuation of anaesthesia). Following the septic insult, the animals are usually not obviously distressed or in pain. Any that appear distressed are promptly culled. We created our Clinical Scoring System following consultation with REDACTED and our named veterinary surgeon to include more clinically relevant characteristics. A pilot study directly comparing the ‘old’ and ‘new’ scoring methodology was performed. The agreement (score awarded to each animal) between biological services and lab staff was improved using the new scoring system. This method awards the highest score from any category (rather than accumulation as previously performed), akin to actual severity assessment that we do for our animals. Scoring is performed at least four times per day and informal assessment is also performed regularly in between. Increasing clinical severity results in more frequent monitoring and additional support such as mashed food, more bedding and supplemental fluids.

Animals scoring three are deemed ‘critical’ resulting in euthanasia on moderate protocols. On severe protocols, we monitor the animals overnight or terminate experiments following three consecutive ‘3’ scores, or two near the end of the working day. All animals that score 4 are promptly culled. The majority of experiments fall under the moderate category where severity limits are strictly imposed using our scoring system, as above. A limited number of experiments will be performed under a severe severity limit. For example, the effectiveness of a treatment for a shock condition is measured by improved survival. Although we generally aim to terminate the study at pre-specified times, we feel there is an important place for some protocols to provide firm verification of benefit. That notwithstanding, we are able to predict mortality resulting in closer, more effective monitoring of ‘critical’ animals.

We intend to perform a more extensive comparison of our clinical scoring methods and aim to publish our results. This could potentially allow further refinement of animal models of sepsis in other UK-based establishments and globally.

The use of circulatory monitoring also allows us to predict mortality through identifying persisting low blood pressure, allowing us to cull the animals electively before they die spontaneously. Monitoring for low blood pressure depends on whether the indwelling arterial line/tether system is being used (and remains patent). If so, during daytime hours

the blood pressure is checked every 2 hours. Animals with a mean blood pressure <60 mmHg are culled as our experience shows that this precedes mortality by a few hours. Echocardiographic assessment of stroke volume (the volume of blood leaving the heart, per beat) at an early timepoint (6h) allows us to predict future mortality (with above 85% accuracy). Similar predictive values can be achieved using heart rate if echocardiography is not available. Predicted non- survivors are more closely monitored and experiments terminated before animals suffer unduly.

Animals lose fluid from their circulation into their tissues as part of the 'capillary leak syndrome' associated with sepsis. Loss of appetite and fluid intake is compensated by infusion of liberal amounts of fluid containing electrolytes and glucose. As noted above, this food can be mashed to aid feeding. Some studies assessing later endpoints in the disease progression are, we feel, justified, as the ultimate test of the efficacy of a treatment for a shock condition is improved survival. There are many examples in both animal models and clinical practice whereby improvements in the circulation, for example, do not necessarily translate into enhanced survival. Thus, while we generally aim to terminate the study at pre-specified timepoints, we feel there is an important place for some protocols to provide firm verification of benefit.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The disease processes of organ dysfunction encountered in critical illness and response(s) to therapies can change with life stage and species. We propose mainly using 8-12 week old animals (opposed to younger animals) as responses observed in this age range more closely mimic the clinical scenario.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

We have a Clinical Scoring System developed in conjunction with Biological Services staff and our named veterinary surgeon. We intend to perform a more extensive comparison of our Clinical Scoring methods and aim to publish our results. This could potentially allow further refinement of animal models of sepsis in other UK-based establishments and globally.

Support for the animals increases in line with the Clinical Score we award. With higher scores we monitor the animals more frequently and provide additional support such as mashed food and pain relief, as required.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We recently took part in a round-table conference with other experts on animal models of sepsis. The outcome was a paper published in three journals informing on best practice. See 'Minimum quality threshold in pre-clinical sepsis studies (MQTiPSS): an international expert consensus initiative for improvement of animal modelling in sepsis'. We will continue to follow these guidelines and keep up to date with literature published during the course of the project.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We keep up to date with, and contribute to, published literature on the refinement of acute and critical illness animal models. We will additionally attend conferences and contribute to workshops set up to promote and inform on the 3RS.

Explain the choice of species and the related life stages

We will predominantly use rats as their metabolic response to infection and other conditions is similar to that seen in humans. Mice show a dissimilar metabolic response as they tend to hibernate, dropping their body temperature and metabolism, in response to some insults. However, we may use them for certain studies (such as mice that are born with an altered genetic background) to test specific biological pathways. The rats we use are typically 8-12 weeks old. Although animals (>1.5 years) rats may be more similar in age to the majority of patients we are ultimately aiming to treat (with additional age-related conditions that can co-occur e.g. high blood pressure and cancer), younger rats show less variation and that means we need to use less of them to prove our biological findings are significant.

A retrospective assessment of refinement will be due by 06 July 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

34. Pathophysiology and Treatment of Neurological Disorders

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	juvenile, adult, pregnant, embryo, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The project aims to advance our understanding of the underlying mechanisms involved in the pathogenesis and chronicity of neurological disorders in the eye or the central nervous system (CNS) and develop potential therapies for patients.

A retrospective assessment of these aims will be due by 04 December 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Worldwide neurological disorders have become the leading cause of disability, and the second leading cause of death. The absolute numbers of deaths and people with disabilities caused by neurological diseases have risen substantially in the past 30 years, and it is expected to increase further as a result of population growth and ageing. The social and financial burdens imposed by these chronic, debilitating diseases include poor quality of life, high health care costs, and substantial loss of productivity. Thus, there is an urgent need to understand the disease mechanisms of these neurological disorders, and develop strategies in disease prevention and treatment for patients.

This project focuses on three of the main neurological diseases: multiple sclerosis (MS), uveoretinitis (this work may be undertaken in the future) and Alzheimer disease (AD). MS is a chronic disease in which repeated episodes of inflammatory demyelination result in irreversible axonal injury in the central nervous system (CNS). MS is a disease of all ages but commonly diagnosed between the ages of 20 and 40. It affects more than two million people worldwide, and the UK has higher MS prevalence than many other countries with around 100,000 people in this country suffering from the condition. MS is the major cause of non-traumatic neurological disability among working adults, it can substantially and adversely affect an individual's quality of life and is associated with high costs for MS patients, their families, and society as a whole. Posterior uveoretinitis (inflammation of the uvea layer, which can involve the retina tissue, thus also called uveoretinitis) often leads to retinal tissue damage and is a potentially blinding condition (10% of blindness in the developed countries) with a significant economic and social impact. In the UK, it is estimated that two to five in every 10,000 people will be affected by uveitis every year.

The number of individuals living with dementia is increasing (more than doubled globally from 1990 to 2016), negatively affecting families, communities, and health-care systems around the world. AD is the most common form of dementia which causes progressive cognitive deterioration and is characterized by beta-amyloid deposits and neurofibrillary tangles in the cerebral cortex and subcortical grey matter.

The direct cause of many neurological disorders is often not known. Currently, there is no cure for MS, uveoretinitis or AD, and the efficacy of the available treatments is limited by many side effects. Thus, better understanding of the underlying molecular mechanisms of these diseases will help develop better strategies to treat the patients and delay disease progression, thus help the patients and families, and reducing the economic burden too.

Experimental autoimmune encephalomyelitis (EAE) and experimental autoimmune uveoretinitis (EAU) are animal models commonly used in research because their pathological features closely resemble human MS and uveoretinitis. EAE provides a valuable tool for obtaining insights into the immunobiology of MS disease and has led to the development of three clinically approved therapies: glatiramer acetate, mitoxantrone and natalizumab. For its part, EAU has contributed enormously to our understanding of uveoretinitis disease and drug development.

Although EAE and EAU are pathologically separate disease models, there is tremendous overlap as both CNS and eye are considered to be the immune privileged sites, and they share similar mechanisms of immunopathogenesis. Furthermore, this has been exemplified by the use of similar, if not the same, biologicals for the treatment of MS and uveoretinitis in the clinic. It should therefore be appreciated that the lessons we can learn

from these two separate diseases will very likely benefit sufferers of either and possibly of other autoimmune diseases.

For AD research, genetically modified animals such as 5XFAD mice have been successfully used to explore the disease pathogenesis, immunomechanisms, and potential therapies. For example, accumulating research evidence has confirmed that AD pathology includes amyloid plaques, neurofibrillary tangles and inflammation in the CNS. In addition, CNS resident microglia cells and immune cells in the peripheral immune system are now known to play important roles in the development of AD. Our studies here aim to improve our understanding of AD pathologies and the interaction between the CNS and the immune cells, which is the key for the development of novel therapeutic strategies for patients.

Research from decades of studies suggests that the dynamic balance of effector and regulatory immune cells, together with their related cytokines/chemokine, control the immune response towards immunity or tolerance respectively, and has a major influence on the pathogenesis of immune mediated diseases including neurological diseases. Thus, factors that regulate the balance of effector and regulatory T cells in the development of MS, uveoretinitis and AD are of particular interest and may represent potential therapeutic targets. However, immune response is a complex manifestation of multi- factorial dysfunction of an array of genes and molecules. Although impressive progress has been achieved in recent decades both in the fundamental understanding of, and therapeutic approaches to these diseases, much still remains to be learnt. The limitations encountered with new therapies would suggest the existence of unrecognised pathways operating in the development of these neurological disorders.

Therefore, it is essential that we understand better the underlying mechanisms of neurological disorders, and better therapeutic control of these diseases, since this will result in huge economic, social and health care benefits.

What outputs do you think you will see at the end of this project?

The project will further our understanding of the underlying mechanisms involved in the pathogenesis and chronicity of neurological disorders, thus informing rational design of novel therapeutic strategies for patients with these diseases, all of which have enormous impact on the economy and the health care of humans.

For example, we are investigating the underlying action mechanisms of immune cytokines in multiples sclerosis, the findings will provide novel insights into the effect of immune molecules on the important immune cell function during the neurological disease development.

The outputs of research using this PPL will include novel knowledge in understanding the underlying immunomechanisms of multiple sclerosis (MS), uveoretinitis, and other autoimmune diseases. The findings will be published in peer-reviewed research journals and presented in national and international conferences (oral and post presentation).

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Neurological disorders are one of the most challenging health problems with global economic and societal impact. The new knowledge we learn from these studies will help

us to understand the initiation and development of MS, Alzheimer's disease and uveoretinitis. In the long term the findings will also lead to the possibility of identifying new molecular targets for autoimmune diseases, for which new pharmaceutical products could be developed for clinical intervention of these diseases.

The information is likely to be of interest to pre-clinical scientists interested in immunobiology. The secondary potential benefit relates to the value of the results to clinicians (ophthalmologist, neurologists) and patients. Furthermore, new molecular targets may be identified, for which new pharmaceutical products could be developed for clinical intervention of these diseases, thus benefiting patients, patient families and the society.

How will you maximise the outputs of your work?

I collaborate with colleagues within the REDACTED, in UK and internationally to promote the research in understanding the immune mechanisms of MS and other neurological diseases. The findings our studies will be published in peer-reviewed journals for other researchers in the field, in addition we will disseminate our findings through presentations at national and international conferences, and thus there are ample opportunities to share data and results with the international community. We also aim to promote public understanding of research in immune mediated neurological diseases via our outreach activities.

Species and numbers of animals expected to be used

- Mice: 4500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Experimental autoimmune encephalomyelitis (EAE) is induced by one subcutaneous immunisation of protein or peptide of central nervous system (CNS) emulsified with complete adjuvant, together with two intraperitoneal injection of pertussis toxin. Experimental autoimmune uveoretinitis (EAU) is induced by one subcutaneous immunisation of protein or peptide of retina emulsified with complete adjuvant, together with two intraperitoneal injection of pertussis toxin. These mice receive prophylactic or therapeutic regimes of treatment (e.g. injection of reagents/cells/biocompatible materials). Mice will then be monitored closely for clinical symptoms (EAE clinical symptoms will be recorded by observation, EAU symptoms will be monitored using fundus imaging). Experiments often last 4 to 8 weeks, and mice are humanely killed at the end of the experiments, and tissues are analysed.

AD models, such as 5XFAD mice, are genetically altered mice; animals start to develop pathology of AD at about 2/3 months' of age. These mice receive regimes of treatment (e.g. injection of reagents/cells/biocompatible materials) before 2 months, or after developing cognitive deficits at around 9 months. Mice will then be monitored closely and

weight will be recorded. Depending on the specific research aims, experiments often last 3 to 6 months, at the end of which mice will be culled and tissues analysed.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

The adverse effect for each genetic modified mouse colony will be dependent on specific genetic modification of the mice. The majority of mice have no gross phenotypic abnormalities, reproduce normally and mature to full age as wild type mice. Some strains have mild phenotypes involving altered behaviour or activity, or susceptibility to disease due to immunomodulation. The information for the adverse effect for each colony will be obtained from the suppliers and/or from literature if published already. Other potential minor adverse effect: Ear notching should involve only slight and transient pain, and no healing problems. Haemorrhage after blood sampling will be controlled by local pressure.

Immunisation protocols for EAE and EAU induction in mice may lead to small skin ulcers at the injection site. However, the small ulcers should heal without the need for treatment and generally cause no discomfort to the animal. Granuloma formation may develop at site of injection when using complete adjuvant and although this is common, it does not appear to cause any health issues in the animal.

Clinically, EAE animals can display a monophasic bout of EAE, a relapsing-remitting form, or chronic EAE. In the monophasic form, disease develops as an acute clinical course followed by complete clinical recovery. Alternatively, animals may develop a stable chronic neurological deficit, or even develop a relapsing/remitting clinical course during which episodes of clinical disease are separated by periods of clinical remission at intervals of 7 to 20 days. Initial recovery is often complete but the disease progression may be associated with the development of irreversible deficits (such as loss of tail tone or hind limb weakness) due to failure of tissue repair. In all cases, after disease onset, animals will be scored on a daily basis and humane end-pionts applied as described above. By contrast, EAU- induced mice do develop retinal inflammation but show no clinical signs.

Animals may also experience transient stress due to anaesthesia used during immunisation or imaging, this is minimised by good technique and the use of short-acting agents.

Normally the adverse effects associated with imaging are not expected to result in any lasting harm. Possible adverse effects include: transient stress/discomfort associated with anaesthesia, dehydration and hypothermia. Repeated anaesthetic sessions may have a detrimental effect on the animals, but this will be minimized by using short-acting inhalation anaesthetics and making the imaging sessions as short as possible. Fluid support will be provided where appropriate. Mice will be kept warm throughout imaging sessions.

Potential adverse effects associated with dosing regimes (e.g. different routes of injection) are not expected to cause any lasting harm, but may include distress or discomfort due to restraint as well as transient, momentary pain during injections. Some signs of illness, e.g. listness, hunching, maybe observed after injection of some of the agents to be used. This is normally transitory (2-48h). Animals will be monitored regularly after injection and if any of the mentioned symptoms persist or deteriorate, humane end-points will be applied as described previously.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The adverse effect for each genetic modified mouse colony will be dependent on specific genetic modification of the mice. The majority of mice have no gross phenotypic abnormalities, reproduce normally and mature to full age as wild type mice. The expected severity is Mild (>95%).

EAE immunised mice are expected to develop clinical symptoms resembling human MS, thus may reach a Severe severity (about 60%). However, control animals without EAE immunisation or animals with EAE immunisation but sacrificed before disease onset will not reach such a level of severity.

While EAU mice develop local inflammation in the eye, this seems to cause little discomfort and animals continue to behave normally. Because a significant proportion of animals in this model will also undergo dosing regimens and repeated anaesthesia for fundus imaging, we assign a Moderate severity to about 60%. Control animals without EAU immunisation or animals with EAU immunisation killed before full disease onset will likely score a Mild severity.

AD mice develop amyloid protein accumulation in the CNS around 2/3 months, then rapidly develop AD pathology. These animals are expected to develop moderate severity with cognitive impairments. Experiments using WT littermates as controls or AD mice completed experiments before clinical symptoms will only experience Mild severity. Therefore 80% of mice are expected to be in Moderate category.

What will happen to the animals at the end of the study?

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 04 December 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The proposed studies investigate the underlying immunopathogenetic mechanisms of neurological diseases (MS, uveoretinitis or AD) and effect of proteins/compounds/cells or other novel treatments on the development of these diseases in normal and pathogenic

conditions of the neuron system and determine the cellular and molecular mechanisms involved. These studies require an animal with an intact immune system and nervous (or eye) system. The complex interplay of cells and cytokine interactions between the eye or the central nervous system and immune organs *in vivo* simply cannot be replicated or replaced by any available *in vitro* models. It is absolutely critical the functional studies are analysed *in vivo*. Mice are the best animals for this work because of their structural and physiological similarities to the human immune system. There is no good alternative model than the use of mice that can answer the specific questions of the proposed project.

What was your strategy for searching for non-animal alternatives?

Whenever possible we will use non-animal alternatives in this project. For example, *in vitro* culture experiments will be used to study the effect of interested reagents on cell toxicity, immune cell activation and neuron myelination etc. Previously we used a myelinating culture system to reproduce myelination *in vivo* and study the role of IL-33 in CNS myelination.

Why were they not suitable?

These *in vitro* experiment systems do not have the complex interplay of cells and molecule interactions between the organs CNS/eye and the immune organs, thus not suitable for research to determine how immune system and CNS/eye system interact with each other during the development of many neurological diseases.

A retrospective assessment of replacement will be due by 04 December 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals will be used in each protocols below are based on the current funding obrained and potential research interests through collaborations.

Protocol 1: breeding of genetically modified mice, 1500;
Protocol 2: EAE induction, 1500; Protocol 3: Animal models for AD, 1500.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

We will take the following measures to ensure that minimum number of animals used in this project:

Use of minimum numbers in each experiment: The experimental designs and methods of analysis of the results have been discussed with the Statistical Services Unit at the REDACTED. Experiments will be designed to use the minimum number of animals per group consistent with obtaining data that can be tested statistically. These numbers are based on our considerable collective previous experience of the numbers required and power projection of numbers required for our future needs. For the qualitative experiments, the amount of material required is the minimum necessary to provide an adequate description. We have also planned to reduce the animal numbers by harvesting multiple organs for analysing.
We will continually review our results to adjust the number of animals used in experimental groups so that we use the minimum number of animals to have statistically valid data.

Use of clinical scoring: Clinical score of EAE is commonly used. For EAU model which may be undertaken in the future, we will use the recently developed non-invasive *in vivo* fundus imaging techniques for clinical scoring of retinal inflammation. This approach enables the evaluation of disease severity in live mice thus reduces the overall numbers of animals required to screen new reagents and to assess their potential and safety for human use.

What other measures apart from good experimental design will you use to minimise numbers?

We will use other measures to optimise the number of animals used in this project:

We will work closely with staff at the animal unit for efficient breeding of mice in protocol 1.

Small pilot studies will be performed for any new project to ensure the success of following *in vivo* experiments and reduce any unnecessary waste of animals. Following that power calculations will be performed to calculate the animal group size for subsequent experiments.

We also plan to reduce the animal numbers by harvesting multiple organs for analysing.

A retrospective assessment of reduction will be due by 04 December 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

This project will use EAE and AD models.

Depending on the antigen used and the genetic make-up of the animal, rodents can display a monophasic bout of EAE, a relapsing-remitting form, or chronic EAE). Most commonly, EAE induced with MOG peptide emulsified in complete adjuvant will used. The model is easy and quick to induce and is a monophasic EAE model, mice recovers from the symptoms after peak stage and thus less suffering.

We use a well established 5xFAD mice for AD disease study. Mice live a normal life for several months then develop pathology of AD, which is important for the studies.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The project aims to investigate the underlying pathogenesis of neurological diseases in the CNS and the eye, and develop potential therapies for patients. The animal models used require the intact complex the complicated immune and neuronal interactions between molecules, cells, tissues and organs *in vivo* which are important in the initiation, development and resolution stages of various neurological diseases. Therefore, *in vivo* work using the proposed animal models is required.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

We use the following steps to refine our experimental procedures:

- The experiments (all using mice) of EAE often last 4 weeks from immunisation day to finish, some will be up to 8 weeks or more to study the long term symptoms of disease.

AD models will be kept for longer period because of the nature of the disease. However all experiments will be kept to the minimum length needed for the experiment to acquire the necessary data.

We use our improved immunisation EAE protocol (cleaning the injection site and needles, prior to immunisation & carefully avoiding the leakage of injection reagents) to reduce adverse effects such as skin ulceration caused by the use of Freund's complete adjuvant (CFA). In the last 5 years, severe skin ulceration has been rare (<5%). EAE mice develop will be provided with soft food and extra bedding when they develop EAE, and will be monitored closely.

We have developed non-invasive *in vivo* clinical scoring methods that allow early stage disease to be investigated and changes in disease severity to be quantitatively analysed, which in turn can helps us refine our end-points.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Guidance from the NC3Rs of animals in research, and advice from RSPCA for laboratory animals.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

I will learn advances in the 3Rs through various opportunities such as the REDACTED annual 3Rs symposium, and information from NC3Rs.

We aim to implement advances in the 3Rs in our research whenever we can. For example, the new recommendation of using one needle per animal, we had a discussion with our NVS and revised our protocol two years ago.

Explain the choice of species and the related life stages

Normal naive mice or mice with genetic modification of specific genes of research interest will be used in this study. The animal models for multiple sclerosis (MS), uveoretinitis and Alzheimer's disease (AD) have been the essential tools used in research in our understanding of the underlying pathogenesis of disease development, and in the recent development of therapies for patients because of their pathological features closely resemble the disease in human.

For research in MS and uveoretinitis, young adult mice are best in developing disease models. For AD model, adult and old mice (up to 9 months) will be used as symptoms resembling AD patients develop at old age mice.

A retrospective assessment of refinement will be due by 04 December 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

35. Pharmacological characterisation of novel therapies

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b)

Key words

Antigenic Challenge, Pharmacokinetic, Breeding, Mutants, Tolerability

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project aims to test and develop novel therapies to ensure they are safe for use and observe how they behave in the body prior to being used in animal models of human disease.

A retrospective assessment of these aims will be due by 15 July 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these

could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

By contributing to the development of new candidate drugs, our project will accelerate the discovery of new treatments, for the benefit of patients and improving their quality of life and reducing symptoms. Understanding the safety profile of novel therapies and the targets they act on will allow for a smoother transition to other studies looking at the efficacy of these drugs on different diseases.

By providing high quality services and scientific expertise, we make the testing of such drugs quick and reliable, ensuring that effective treatments are identified at the earliest opportunity. This means benefits to patients are realised in a timely manner and potentially harmful or ineffective therapies are identified long before they get to the stage of being given to people.

By providing new disease models we enable a greater understanding of a wider range of diseases, allowing for more treatments to be tested with the potential to go further. Disease models need to be predictive of the human model, so these models are constantly improving, requiring appropriate validation.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

This project will use rats and mice. The estimated number of animals to be used over the duration of the project is 6500.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

Animals may experience moderate distress as a result of the procedures. Animals will be closely monitored and any animals experiencing more than moderate effects over a prolonged period will be humanely killed. At the end of an experiment, all animals will be humanely killed. The mechanism of action of some compounds may lead to transient adverse effects due to, for example, cytokine storm. Increased monitoring will be implemented as required.

A retrospective assessment of these predicted harms will be due by 15 July 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

These studies are required to understand how drugs behave in an animal prior to moving into clinical trials.

The complexity of the pharmacological response cannot be wholly reproduced *in vitro. In vitro* experiments on cell lines and ex vivo experiments on cell cultures will be performed. However, the limitations of these methods do not allow them to replace the use of experimental animals: there is no alternative to the use of a living animal that would allow the objectives to be met.

A retrospective assessment of replacement will be due by 15 July 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

The number of animals used will be the minimum required to ensure meaningful data is acquired. Experiments will be carefully designed to make sure that valid results can be obtained using the smallest number of animals.

Use of IVIS will help reduce the number of animals required by doing in-life assessment of biodistribution and duration therefore minimizing the number of possible satellite groups required for sampling tissues at different timepoints.

A retrospective assessment of reduction will be due by by 15 July 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Animal suffering will be limited by ensuring the most appropriate, robust and well-defined models are used in order to achieve the objective. Mice and rats will be used for the projects in this licence. Mice with changes in their genes may be used to mimic some human diseases.

Animal suffering will be limited by ensuring that the models used cause the least amount of harm to the animals. The mildest dose will be used, and studies will be kept as short as possible. Animals are monitored frequently for signs of discomfort, and appropriate action taken promptly. We will monitor animals closely throughout the studies, and they will be treated or humanely killed if they develop signs of excessive suffering.

Animals are housed in groups and kept in an appropriate environment with plentiful bedding and nesting material and suitable object that allow them to express normal behaviour. All staff are trained in good animal handling procedures. Animals are always handled gently and humanely, especially animals which may be in pain. Animals may be acclimatised to being handled prior to the experiment starting so that they are less stressed once the study begins.

A retrospective assessment of refinement will be due by by 15 July 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

36. Platelets in haemolytic diseases

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.
What is the aim of this project?

The aim of the project is to understand the role of platelet's adhesion receptors in sickle cell disease and their contribution to inflammation and thrombosis. Platelet receptors involved in platelet activation also regulate immune cell activation and inflammation.

A retrospective assessment of these aims will be due by 27 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Classically, platelet are known for their role in haemostasis and thrombosis. However, recent work have shown their contribution to the inflammatory response, organ damage and wound repair. We have identified key receptors on platelets in particular CLEC-2, GPVI, CLEC-2 and TLR4 as key regulator for these activities using mouse model of lipopolysaccharide-induced endotoxemia and bacterial infection. These studies were performed in wild-type mice with no specific alteration or susceptibility to infection. In this licence, we will investigate the role of these receptors in a humanized mouse model of sickle cell disease, a haemolytic disease associated with increased susceptibility to infection. Therefore, targeting platelet receptors in this model might represent a unique pathway to decrease the severity to infection in sickle cell disease.

What outputs do you think you will see at the end of this project?

The aim of the work is to investigate the potential of targeting platelet adhesion receptors in inflammation and thrombosis in haemolytic diseases. The use of clinically relevant mouse models are critical to assess to role of platelets, identify targets and validate in vitro observations for selected drugs. The information generated will be published in peer reviewed scientific journals and can be used to inform clinical trials with the selected drug(s) in patients with haemolytic diseases. This would be undertaken in collaboration with clinicians at local teaching hospitals. It is hoped that if effective as the drugs to be tested in are already approved and used for other diseases such as Leukaemia and Malaria they could rapidly progress to human trials.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

In the short term, the data generated in this project will establish the role of platelets adhesion receptors in sickle cell disease. In the absence of key data on the role of platelets in sickle cell disease, it is currently not possible to assess the role of anti-platelet drugs. Therefore, using a humanized mouse model of sickle cell mice will clearly define the role of these receptors in thrombosis and inflammation in sickle cell disease. This will inform the direction of future research that we disseminate to scientists and medical doctors through conference presentations, and publication of the data in scientific journals using the ARRIVE guidelines on animal experiment reporting.

On the long term, based on the results, selective drugs targeting platelets can be administrated, in particular drugs available on the market that can be re purposed to use in haemolytic patients.

Sickle cell disease patients develop pain crisis, with no available treatment. Identifying a target that can reduce thrombosis and inflammation is crucial to justify a clinical trial that can reduce pain in patients, decrease organ damage and increase survival in these patients.

How will you maximise the outputs of your work?

The information generated in this work will be published in peer reviewed journal. Even in the absence of beneficial effect of anti-platelet drugs or immunomodulatory molecules on thrombosis and inflammation, this information is needed to increase the knowledge about the cause of thrombosis and potential targets. Moreover, I collaborate with many experts in the field in haemolytic diseases, who will give advice on possible changes that will be required to study these diseases in mice and compared to patients.

Species and numbers of animals expected to be used

- Mice: 1500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Terminal procedures will be undertaken under non recovery anesthesia where the animals will only be aware of the anaesthetic being administered and may experience mild distress and no pain. Procedures using infectious and inflammatory agents will also be undertaken under non-recovery anaesthesia.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Substances will be delivered under terminal anesthesia.

As sickle mice have progressive organ damage, they will show increase in the clinical disease over time.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Sickle mice show impaired organ function.

Around 50% of mice used on the licence will develop the disease (Sickle mice) and 50% of mice have normal phenotype (Non-sickle mice).

Non sickled and sickled mice will be injected with substances under terminal anesthesia and will not experience pain or suffering from the procedure.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 27 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We are not able to monitor inflammation and thrombosis leading to organ damage under haemolytic conditions in the laboratory. Our main output is assessing organ injury and thrombosis in multiple organs, which cannot be replicated in vitro because of the complicated nature of the interrelationships involved in these processess.

What was your strategy for searching for non-animal alternatives?

The fundamental reason why the use of animals is required to understand these processes is that at present no methods in the laboratory exist to model hemolytic diseases. Therefore, many experiments will be performed in vitro but the use of animal work of crucial to assess the relevance.

We are currently working on developing organoids to mimic the complex environments in different organs. If we are successful, many of the animal work will be tested using organoids and the use of animals will be further reduced.

Why were they not suitable?

As mentioned above, sickle cell disease are highly complex multifactorial diseases that cannot be mimicked in vitro. Although some studies can be done in vitro, the clinical relevance cannot be observed in vitro or ex vivo. Therefore using animals of hemolytic dieases are crucial to understand the underlying mechanisms.

A retrospective assessment of replacement will be due by 27 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The approximation of the sample size for in vivo experiments was done by the G*Power 3.1.3 software. In HUS, the major read out is kidney function assessed by blood urea nitrogen (BUN). Based on my preliminary data and the literature, a sample size of 8 per

group would provide 80% power to demonstrate a difference of at least 50% in one of the major parameters of interests (BUN). A similar sample size will be applied for SCD. For intravital microscopy, based on published data and our previous experience, the sample size for the intravital microscopy experiments, based on 70% effect size, α error probability taken as 0.1 and desired power value of 0.80, gave a value of 6 mice per group. For platelet functional assay, based on our previous experience, 5 mice are required to assess platelet aggregation and protein phosphorylation. For microfluidic assay, 4 mice are required per experiment to test the inhibitory effect of drugs. Two-way ANOVA with Bonferroni's multiple comparison test will be used to compare the effect of platelet receptor deficiency or drugs on kidney and lung function. Mean differences in other parameters will be analysed by either Student's t-test (two groups comparison) or one-way ANOVA with Bonferroni post-hoc test (more than two groups) using GraphPad Prism software.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

As mentioned above, the numbers were calculated based on our previous experience and based on power calculation.

Reduction will be achieved by first performing experiments on human platelets and immune cells through the use of pharmacological inhibitors to identify proteins of interest. All inhibitory will be first tested in vitro using ex vivo flow assay to perfuse blood on endothelial cells before injecting in mice. Only receptors that show clear role in flow assay will be tested in mice.

We have refined our techniques for use of small amounts of blood from mice. This has involved development of new tests that require very small numbers of platelets, such as flow cytometry and static adhesion assays. Thus, through the combination of this experience and allocation of people, we are able to keep the numbers of mice to the minimum required to answer a particular question.

What other measures apart from good experimental design will you use to minimise numbers?

Statistical analysis to ensure that we use the minimum number of mice per group that will be informative will be performed.

We are using a staged approach, involving pilot studies to ensure that an appropriate number of animals are to be used.

To maximize the information gained from a single animal we aim to take samples from the blood under terminal anaesthesia and then from multiple body sites post mortem.

A retrospective assessment of reduction will be due by 27 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

The vascular and immune system of mammals is highly conserved with cell types and mechanisms well-maintained. The mouse has been selected because of established and reliable transgene technology and extensive literature on sickle cell disease and haemolytic uremic syndrome (a collection of diseases that involve destruction of red blood cells and inappropriate clotting which leads to kidney damage) models in murine strains with established and reproducible protocols due to the reliable reagents available.

In SCD mice, we will use a behavioural clinical score indicative of the extent pain and distress that each animal is experiencing to help ensure humane endpoints are maintained. In addition, pain relief and hydration will be maintained throughout the protocol to reduced pain and suffering. Blood count will be a good marker for VOC development.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The aim of this study is to assess the role of platelet and immune cell interaction in disease development and progression. It is therefore not possible to perform this on terminal anaesthetised mice. However, their response to inflammatory response in the presence of anti-platelet drug, which is the most severe protocol, will be performed under non-recovery protocol.

We cannot use non-mammalian species for this work, as mammals are the only animals to have platelets. In mice there is established and reliable genetic alteration technology, and established tests of platelet function. There are a large number of genetically modified mutant mice available and there is extensive amount of work that has already been performed and published using mouse models

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Our procedures will be performed under terminal anesthesia.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment.

Experiments will be conducted in accordance with the guidelines published by the Laboratory Animal Science Association (LASA).
The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE guidelines

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We will stay informed by advances in the 3Rs through attendance of seminars and conferences, as well as discussions with the NVS and NACWOs.

We will review each experiment on completion to determine any refinements that can be applied to future experiments.

Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially used to replace animal use.

We will use SyRF the free online platform for researchers to perform a systematic review and meta- analysis of animal studies. https://www.nc3rs.org.uk/camarades-nc3rs-systematic-review-facility-syrf

We will also stay up to date with guidance published by the International Society for Thrombosis and Haemostasis (ISTH) Scientific and Standardisation Committee on the most refined experimental methods for haemostasis and thrombosis research.

Explain the choice of species and the related life stages

Mice are the animal of choice as there are a large number of genetically altered mice available and because of the knowledge built up over 20 years of research. We will use adult mice, usually between 6 – 16 weeks of age.

There is no other model of sickle cell disease that can be used. The major problem in SCD is progressive organ damage. This cannot be monitored in vitro. Moreover, the use of humanized SCD mice are the closer model to the human disease.

A retrospective assessment of refinement will be due by 27 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

37. Preventing SARS Coronavirus 2 infection and disease

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	juvenile, adult, aged
Hamsters	juvenile, adult, aged
Ferrets	juvenile, adult, aged
Marmosets	juvenile, adult, aged
Rhesus macaques	juvenile, adult, aged
Cynomolgus macaques	juvenile, adult, aged
Tamarins - red bellied (Saguinus labiatus)	juvenile, adult, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

To establish appropriate models of infection with SARS Coronavirus 2, so that the mechanism of preventing infection or disease may be established and effectiveness of potential treatments evaluated.

A retrospective assessment of these aims will be due by 02 December 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The immediate global threat of Covid-19 caused by SARS Coronavirus 2 cannot be underestimated. More than 20,000 deaths have been recorded in the UK to date and worldwide, it is possible that many millions may die as a result of SARS Coronavirus 2. It is critical that suitable animal models of SARS Coronavirus 2 infection and/or disease are identified and developed very quickly, so that candidate vaccines may be evaluated at the earliest opportunity. This will ensure that the most promising candidates are selected rapidly for further development. Previous studies trying to develop vaccines for other Coronavirus infections of man or cats have found that immunisation with some candidates may not prevent infection, but may make the disease worse. We must identify whether this is the case for SARS Coronavirus 2 and identify the mechanism of how to prevent infection and avoid making the disease worse.

What outputs do you think you will see at the end of this project?

We expect to publish scientific papers that will be published in open access journals describing the studies that have been undertaken and the ensuing results.

We anticipate that these data will guide the development of documents that provide medicines manufacturers and regulators with a framework for performing safety and efficacy tests for products designed to prevent or treat infection with SARS CoV 2 which causes Covid-19

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

It is anticipated that the attainment of intermediate Objectives 1 and 2 will generate data that will establish the most appropriate model for evaluation of infection and or disease. This will impact on medicines manufacturers and regulators within a year of initiation of the project by providing information on the appropriate laboratory animal model that they should apply to ensure that candidate medicines are both safe and effective.

The completion of Objective 3 will provide a go/no-go decision on the progression of a candidate antibody-based medicine into clinical trials in the UK

In the longer term the attainment of Objective 4 will provide a robust framework for developing superior assays that apply the principles of the 3 R's in achieving the same safety and efficacy data.

How will you maximise the outputs of your work?

We shall collaborate with scientists at other establishments in the UK and elsewhere. In some cases we shall receive candidate materials from others for pre-clinical evaluation in the model systems established at this site. In other cases we shall share information at an early stage on our planned studies and also early results with other establishments with

equivalent facilities to ensure that each programme of work does not duplicate but complement each other.
We anticipate that engaging with appropriate international teams dealing with Coronavirus outbreak and under the auspices of WHO will enable our data to have early global impact, whether it is positive or negative.

We will work closely with national regulators and medicines/vaccine developers to share our knowledge and information at the earliest opportunity.

We shall publish our work, initially in pre-print on line platforms and then in peer reviewed open access journals to share our data at the earliest opportunity.

Species and numbers of animals expected to be used

- Mice: 250
- Hamsters: 125
- Ferrets: 80
- Marmosets: 40
- Rhesus macaques: 80
- Cynomolgus macaques: 80
- Other non human primates: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

In initial studies, animals will be administered a specific amount of virus onto the nose, into the trachea or onto the surface of the eyes to mimic the routes of transmission of SARS CoV 2 between humans. Blood and oral and nasal swabs will be collected before and after administration of the virus. These will be analysed for the presence of the virus and/or antibody or other responses made by the animal to the virus. Once the most suitable species is identified for further study and the timetable of virus detection and control is determined, then animals will be treated with a candidate treatment such as a vaccine or an antibody-based medicine before being administered with the virus. These studies will last no more than 4 months with the virus infection part lasting just over a month.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Some animals may get coronavirus disease, but the evidence from studying related corona viruses is that the disease in most animal species is relatively mild. Treating animals prior to infection will be similar to people being vaccinated - a short pain for the duration of injection. There is a small risk that some vaccines and treatments may make the subsequent SARS CoV 2 infection worse. If so then there may be fever and weight loss - weight loss may be controlled/slowed by providing supporting husbandry measures

eg easily consumed food. These would normally be expected to last a couple of days and then recovery observed. If this is not the case then animals will be killed humanely.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Most studies will be classed as Moderate Severity. In most cases this will be because of the needs for animals to undergo surgery to allow implant of a remote telemetry device that allows continuous monitoring of body temperature and movement. This device, which is about the size of two kidney beans stuck together will be implanted under the loose skin in the scruff of the neck/back, where they are well tolerated by ferrets and non-human primates. A much smaller device is used in mice and hamsters.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 02 December 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

In order to understand the basic cellular and molecular pathology of infection and complex multi-organ host responses, it is necessary to use laboratory animals. Furthermore, the complexity of host immune responses to experimental treatments including candidate vaccines or antibody-based medicines, cannot be fully reproduced in in vitro systems. The urgency for identifying safe treatments or vaccines requires rapid action. Waiting for results from other groups to be shared in order to establish whether it may be possible to model aspects of this programme of work in vitro or in silico would introduce delays in development of safe effective treatments. This would lead to people dying from Covid-19, perhaps unnecessarily.

What was your strategy for searching for non-animal alternatives?

In vitro recapitulation of susceptibility of different cell types to virus infection. In vitro methods of recapitulating induction of immune responses.

Why were they not suitable?

Neither methods provide the appropriate combination of cell types in the 3D structure found in the broad range of organs to either fully mimic the situation in vivo

A retrospective assessment of replacement will be due by 02 December 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimate is based on each species being suitable as a model for SARS-CoV 2. However, as described previously, it is not envisaged that all species will be taken forward but the most appropriate for the scientific questions that are being addressed.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Initially the concept of performing a pilot study rather than large group study will enable an indication of the species which are susceptible, and the comparative course of disease resulted in a significant reduction in the number of animals or all species. Subsequently, the selection of only up to 3 specific laboratory animal species for more detailed analyses, based on similarity of infection kinetics and host responses to those of humans (including expression of disease symptoms) will reduce the total number of laboratory animals required. It is not anticipated that experimental treatment studies will need to be performed in more than one species, unless there is a likelihood that they may yield markedly different outcome (for example a vaccine or treatment that might protect in one species but not in another). The group will use the regular flow of pre-published reports from other groups around the world to guide experimental design, prioritise studies and prevent duplication. This information will be gathered from regular telecoms coordinated by international organisations such as WHO and CEPI.

What other measures apart from good experimental design will you use to minimise numbers?

If fewer than 3 species prove to yield robust models of infection and disease with SARS CoV 2, then the 1 or 2 suitable models only will be studied. In addition, to good experimental design for a single experiment, where a series of challenge studies are planned then committing to the use of a common challenge stock of virus or a positive control treatment, will enable positive and negative controls to be combined from each stand alone study increasing the statistical robustness of the studies.

A retrospective assessment of reduction will be due by 02 December 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

At a time when we are responding to a new infectious agent causing large numbers of deaths worldwide, we need to identify the most appropriate models for studying disease and establishing the risks and benefits of treatments quickly. Therefore, it is proposed that Mouse (normal and human receptor expressing) hamsters, ferrets, New World and Old World monkey models should be compared and provide head to head analysis of infection and disease. Using the data generated then selected models will be developed further.

The use of remote measurement of body temperature and movement will minimise the amount of animal handling required and yet important and frequent measurements of key disease markers.

It will not be possible to administer analgesics during the first 7 days after Step 5 of the protocol as fever is recognised as a key disease symptom which we would wish to recapitulate in a laboratory animal model. However, if fever lasts more than 48 hours or increases throughout this time, then analgesics will be permitted.
All subjects will be killed humanely at the end of the experimental protocol

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

A range of laboratory animal species will be compared and selected species taken forward. There will be an endeavour to include less sentient species for more detailed studies (particularly when larger number of experimental animals are required to evaluate potential medicines or vaccines). However as most studies last more than 2 weeks it is not possible to use terminally anaesthetised subjects.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Small animals are habituated into groups for 1+ weeks prior to entry onto study. Non-human primates are grouped according to information supplied by breeding organisation and habituated at our establishment for 2+ weeks prior to entry onto study. The application of remote telemetry to this study (for ferrets and non-human primates) is based upon

experience gained from another PPL, where improved methods for surgery of implantation have been evaluated and applied. Early studies will establish the time-course of disease symptoms following infection. Once these are established, then the optimal timetable of treatment with analgesics that minimise suffering without interfering with scientific purpose of the study will be determined and applied going forward. Furthermore the timetable when more frequent monitoring of experimental subjects will also be identified and applied in future studies.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

FELASA guidelines will be applied to guide dose volume/route per laboratory animal subject. NC3R's documents on husbandry and housing and procedural work will be met or exceeded.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Within the field of Coronavirus work, I am linked into WHO and other scientific groups to hear of their scientific progress on the development of models. Through our network within our organisation, internally and externally developed improvements in 3R's are routinely shared to all PPL holders and these will be incorporated into the Coronavirus work as appropriate.

Explain the choice of species and the related life stages

At this present time, when there is an urgent need to develop safe and effective treatments, we need to evaluate the widest range of species of laboratory animal for which there is evidence, from other Corona viruses, that they may prove useful as a model of SARS CoV 2. Because there is clinical evidence that older people are at greater risk of severe disease, it is prudent that, for each laboratory animal species, old animals are compared with juvenile/adults of the same species. This may result in specific factors, that co-associate with age, being identified as risk factors for severe clinical Covid-19.

A retrospective assessment of refinement will be due by 02 December 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

38. Redacted

Project duration

5 years 0 months

Project purpose

- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Cattle	juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The aim of this project is to generate larvae REDACTED. This is done by working with a natural host (cattle) in whom many larvae can be cultivated and harvested from the faeces of very few animals. The larvae are later attenuated and used to produce a vaccine which protects cattle against REDACTED. Generation of larvae for the vaccine is known as 'primary production'. The resulting vaccine is well established and widely distributed.

A retrospective assessment of these aims will be due by 27 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

REDACTED is a common disease of cattle in temperate regions of Europe. Clinical symptoms usually begin with coughing and raised respiratory rate and effort. In extreme cases, severe respiratory distress and death can occur. As sexually mature worms develop in the bronchi and trachea, animals suffer bronchopneumonia and acute obstructive pulmonary disease of varying severity depending on infective dose, general condition, climate and opportunistic secondary infections. Clinical signs may abate as active immunity develops but lasting and irreversible damage may have occurred to the respiratory system. During infection, wellbeing and productivity of infected animals are significantly compromised.

Husk outbreaks are not predictable from year to year. Build-up of infective larvae on pasture can occur very rapidly in optimal conditions and severe disease can occur rapidly in the absence of immunity. Although modern anthelmintics can control, treat and suppress REDACTED, they only act while the active ingredient is present within the body and offer no immunity. There is also increasing concern over anthelmintic resistance and their usage should be minimised. There is demand for vaccination. The vaccine is the most logical and environmentally friendly method of controlling this disease and offers benefits for the health and welfare of cattle and calves at a time when (out at pasture) they may be exposed to the parasite and be clinically monitored infrequently. Enough larvae to satisfy the entire global demand for vaccination is produced from approximately 30-40 calves per annum. Therefore the cost:benefit analysis is highly favourable.

What outputs do you think you will see at the end of this project?

It is expected that for each year this project remains active, the global demand for REDACTED shall be met.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Vaccination with this product is extremely effective. Therefore, given the predicted demand during the life of the project, approximately 2 million cattle shall directly benefit from protection. This helps to preserve their wellbeing, optimise milk and beef yields on farm and reduce reliance on anthelmintics in response to REDACTED outbreaks.

How will you maximise the outputs of your work?

Close collaboration with colleagues within the organisation has identified potential ways to increase recovery of larvae from raw harvest. This may mean more vaccine can be made, and/or fewer calves can be worked with. There are concepts to be able to extend the shelf-life or get the product to market with more shelf-life remaining. This creates the potential to look to new markets and increase the sales and protect more animals. It is entirely feasible that the above are achieved with no increase in animal usage.

Species and numbers of animals expected to be used

- Cattle: 500

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Calves are appraised and possibly blood sampled on farm from one day of age onwards, and not commonly sampled on farm beyond two weeks of age. This sample may be screened for acceptance criteria to bring to site.

Suitable calves are brought to site whereupon occasional samples including faeces and blood are taken to assess presence of certain pathogens. Calves are enrolled on a comprehensive vaccination programme for good herd management which is outside of the scope of the Act. Vaccinations are commonly a combination of injectable or intranasal administrations.

Calves may also be subject to a bTB comparative intradermal test for regulatory bTB testing.

Currently a similar test may also be conducted for Johnes disease.
The above procedures occur during the rearing phase i.e. before infection with REDACTED larvae. During the rearing phase it is unlikely that animals will be singly housed. Single housing shall only be applied for husbandry purposes e.g. to monitor an ailing animal, allow recovery from injury or disease, or where mixing delivery cohorts to avoid single housing could be more aversive than remaining singly.

Shortly prior to infection, calves are appraised by a vet for suitability to undergo larvae production.

Suitable calves are infected with a small aqueous oral dose of a known number of viable infective REDACTED larvae. Approximately 7 days later, calves are rehoused to closely monitor and treat animals as necessary. Preliminary appraisal of larval output via faecal sample and (modified) Baermann test begins at patency (approximately 21 days post infection) and continues frequently throughout production. At approximately 24 days post infection a harness is fitted to each calf to allow them to acclimatise to it. At approximately 26 days post infection a collection bag is suspended from each harness and faecal collection begins. Collection bags are changed several times daily. Collection will continue until a humane or scientific end point is reached.

From larval challenge onwards, calves are appraised and clinical signs recorded e.g. rectal temperature, respiratory rate and effort at least daily. Veterinary treatment or euthanasia at the humane end point are aided by these assessments. Therapeutic treatments to alleviate clinical signs may be administered by or under the direct control and guidance of the NVS or veterinary clinician.
Continuation of treatments may be delegated by the veterinarian and administered by experienced animal care staff.

Animals are likely to be housed singly during production. This aids administration of therapeutics and collection bag changing which could be distressing if having to isolate frequently from a larger pen to perform these tasks. Conspecifics may also interfere with collection bags and reduce faecal/larval recovery which is detrimental to the project.

No calves which have been infected with REDACTED shall be considered for release from the Act, and they shall be humanely euthanased. A blood sample may be recovered post-euthanasia for a further screen.

Other typical husbandry procedures outside of the scope of the Act may occur during the lifetime of the calves in REDACTED's care which include:

transportation by road temperature monitoring weighing castration disbudding

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

During the rearing phase there are not likely to be any adverse effects of procedures applied. Sampling routes and techniques are mild. There is potential for transport stress and/or related disease. Healthy calves are transported straight from farms of origin to site and are given electrolyte treatment shortly after arrival to guard against dehydration and transport stress. Other treatments are unlikely and only given if prescribed by a vet.

As in all calf rearing enterprises there may be losses from non-study-related diseases e.g. calf scour and joint ill. These will be treated appropriately wherever possible but calves not responding to treatment may be euthanased.

Adverse reactions to registered vaccines rarely occur. If seen it consists only of painless or mildly aversive swelling around the injection site. No treatment is necessary in these instances.

The act of infection, faecal sampling and harnessing of calves is no greater than mildly invasive or aversive.

Calves infected with REDACTED can, at worst, develop severe ill health and may die. Hyperpnoea and tachypnoea are seen. At least daily examination of each calf from approximately day 7 post infection is required, along with appropriate treatment which enables most calves to tolerate the infection reasonably well. A few may be naturally refractory to infection, but the majority will require regular treatment.

Some discomfort is created by giving frequent injections for treatment. The greatest frequency for these is likely to be between days 7 and 21 post infection. Clinical signs usually settle down once patency is reached so the number of injections is reduced and kept to a minimum using long acting or enteral preparations wherever possible.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Approximately 30% shall reach only mild severity
Approximately 50% shall reach moderate severity
Approximately 20% shall reach severe severity
These figures include those calves reared as 'back-ups' with the expectation that they shall not be worked with for production. The incidence of work with back-ups is minimal at

<0.5% of all calves worked with in production in 8 years. This last occurred when just 2 calves were used in 2011.

What will happen to the animals at the end of the study?

- Rehomed

A retrospective assessment of these predicted harms will be due by 27 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

REDACTED are highly host-specific and cannot reproduce outside of a host. Therefore in order to produce a vaccine from attenuated REDACTED larvae, a small number of bovine calves are infected with REDACTED which are allowed, under controlled conditions, to go through their natural life cycle. This process generates enough larvae from approximately 30-40 calves per annum to satisfy global demand for the vaccine.

What was your strategy for searching for non-animal alternatives?

Although research into ways of producing an effective vaccine in vitro have been conducted by several institutions and REDACTED has supported that work, to date none have been successful and hence there is no alternative than to work with calves to produce the larvae.

Dictyocaulus viviparus can survive in alternative hosts, but these are not as suitable for large scale production as bovine calves.

Why were they not suitable?

Guinea pigs can support small *Dictyocaulus vivaparus* larvae infections under experimental conditions but cannot support the maturation and reproduction of the parasite; therefore cannot supply larvae for production.

Deer and llamas can harbour *Dictyocaulus viviparus* infections but the faecal yield would be low and the animals far harder to source and manage than bovine calves.

A retrospective assessment of replacement will be due by 27 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The vaccine has been produced in much the same way for many decades. Vaccine sales have fallen slightly over recent years however the projection is that there will be some increase during the life of this licence. The number of animals worked with in production will likely be unchanged despite the predicted increase in vaccine sales, however an overall increase in animal usage is being proposed for this project compared to the previous license because of the reporting of procedures applied on-farm (POLEs) where historically these were omitted. The increase is 100% compared to the previous license on the assumption that approximately half of the number of animals sampled are acceptable to be worked with. It is anticipated however, that due to plans to refine the supply of animals, the vast majority of animals will be acceptable and the increase not be required. It must be considered however that the preferred supplier(s) may be lost at any time due to disease, movement restrictions, business decisions by them etc. and the process of finding and screening new suppliers/animals be conducted. In this case, it is reasonable to assume that many more calves may be sampled than 'normal' and these procedures be returned thus increasing actual usage.

Ways to recover more larvae from the raw harvest (without changing the infection dose or in-vivo process at all) have been identified. Therefore, it is predicted that more larvae can be recovered from the same number of animals to accommodate any increase in sales. Furthermore there is a degree of overproduction. Some contingency is essential to absorb any QC batch rejections etc. but the growth in sales which is predicted shall absorb some of the overproduction.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

The number of animals worked with has decreased proportionally to the number of vaccine doses being sold in recent years, and is currently well balanced. It is being proposed that refinements in the Secondary Production process may help to recover more larvae from raw harvest, which in turn could reduce the number of animals worked with, and/or enable more product to be made for no increase in animal numbers. It is also important to remember that there needs to be a degree of over-production of larvae to account for losses prior to vaccine blending, and output of larvae is not predictable for each animal so it would be a risk to reduce numbers too low.

The current team are committed to investigating potential animal reduction possibilities but it should be remembered that changes affecting the production process could be subject to official change controls or variations, which can take time.

What other measures apart from good experimental design will you use to minimise numbers?

Good communication with product marketing is key to knowing how much product is required for sale. It is possible to work back from that and estimate the number of animals required. To give enough time to rear calves we are always working to a sales prediction e.g. we need to know before we procure calves in the spring, how much vaccine will be sold the following year; because it is that which those calves will be working towards. The sales predictions included in this license address this although there is potential for change due to market forces.

A retrospective assessment of reduction will be due by 27 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

We will work with approximately 30-40 male dairy or dairy cross-breeds per anum at approximately 5 months of age at point of infection. They will be reared on-site from approximately 2 weeks of age. Prior to infection no adverse effects are expected. The infection process is minimally invasive but the effect of infection could be severe. Severe signs are ameliorated as much as possible by a non-specific treatment regime applied by vets or experienced animal care staff throughout the infection process with a higher likelihood for these up to and around patency.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Other species do support *Dictyocaulus viviparus* infections, but it is not feasible to work with them in a scale necessary for production of sufficient larvae for vaccine manufacture. Such species include:

Guinea pigs
Llamas
Deer
The yield of larvae from these species per animal is far lower than that of bovines so proportionally more would need to be worked with. Supply of these species has not been evaluated and may be problematic, and their housing and management systems are not as conducive for faecal recovery. The availability of vaccines and/or therapeutics to ensure high respiratory (and other) health status plus offer treatments during the REDACTED infection or any other intercurrent illness is lacking.

The question of sentience is complex, but it is known that during the production process bovine calves can tolerate the infection well and produce a large yield of larvae. Other species may be considerably more averse to the process.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Where samples are required from the animals (blood and faeces), sufficient is taken in one brief session to perform several tests with the samples. Although these procedures are mild and minimally invasive, the overall number of procedures is reduced by adopting this practise.

Likewise, to reduce the frequency of disturbance when offering vaccinations, a vaccination schedule is very carefully planned to use multivalent vaccines where possible to reduce number of injections, plus combine as many compatible products into one vaccination session as possible.

During production, animals are scored and only treated when considered necessary by vets and experienced animal care staff. Where possible, long acting and/or non-injectable preparations are given to reduce the number of injections.

Calves have access to an excercise yard for a large period of their production process which enables excercise and socialisation. If not group housed, animals are always within close proximity, sight and sound of eachother. Environmental enrichment e.g. scratching posts and mineral licks are available throughout.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

The Code of Practice for animals worked with under A(SP)A is adhered to at all times.

The process undertaken is not experimental and is already well refined and established having been in existence for many decades. The process is limited to a degree in the way it can be changed by the coverage of GMP accreditation, to which alterations require lengthy variation. To this end there are prescribed processes for much of what is entailed, and these are controlled by localised standard procedures which must be followed.

Should there be a lack of published guidance, the afore mentioned dedication of vets and animal care staff to discover and apply best practise which they may encounter or develop shall demonstrate the commitment to refining the process as much as possible.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

The support network including several vets and other named persons in the PEL help to disseminate information posted from bodies such as the IAT, Home Office, NC3R's, RSPCA, UFAW, LARN, LASA etc. Having attended a number of events sponsored by these parties, and being a member of the IAT, I also directly receive mailings, notifications and invitations. Frequent updates are effectively disseminated within our organisation by the HOLC, and by all present at regular AWERB meetings.

The vets being worked with for this project are members of the Vets On-line Email (VOLE) network and British Cattle Veterinary Association (BCVA). Through these plus extensive experience in their roles, they have close connections with the veterinary community. Within these networks, important issues are highlighted, questions raised, connections made, and solutions suggested. It is a valuable resource. Farming publications are also received to the site in which welfare practises for cattle, rarely covered by the afore mentioned parties, are publicised.

The animal care team on site are active in investigating and applying new initiatives for the animals they care for. Some are connected by lineage or community to the farming fraternity and a degree of discussion into available technologies to enhanced cattle welfare does emanate from this source. It is considered a key in-house appraisal parameter of animal care staff that they demonstrate and apply continual improvements to animal welfare. Staff are keen to attend events where there is opportunity for education and networking which may enhance our shared commitment to our animals.

Explain the choice of species and the related life stages

Male calves of dairy or dairy/beef crosses at approximately 5 months of age are the most suitable species for production of REDACTED larvae.

Cattle (from 8 weeks of age) are the target species for the vaccine and working with them to produce larvae yields a significant number in a manageable way. The larvae are recovered from the faeces of the animals and male bovine anatomy is conducive to achieving a 'pure' faecal harvest where the same process with females would lead to urinary and uterine contamination of the faeces. This would make the faeces unmanageable and increase the risk of pathogenic contamination.

The calf's body mass and lung development need to be optimally proportional to tolerate the infection and generate larvae. The process has been conducted in much the same way for many decades, and it is known that a dairy animal reaches optimal target weight of approximately 150-190kg at around 5 months of age. At an equivalent stage of lung development, a beef animal would have a greater body mass which would place greater demand on the respiratory and other physiological systems during an infection. Therefore, they could incur a more adverse reaction than that of a dairy animal. When infected with REDACTED under controlled conditions dairy breeds are most likely, along with careful management from animal care staff and vets, to tolerate the infection and produce acceptable numbers of larvae for a long enough period to satisfy demand for vaccine manufacture.

A retrospective assessment of refinement will be due by 27 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

39. Primate Neurobiology of Cognition Informing Neurology and Neurosurgery Patient Work

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

learning, memory, primate, prediction, neuroscience

Animal types	Life stages
Rhesus macaques	adult
Marmosets	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Uses non-human primates
- Contains severe procedures
- Required at inspector’s discretion

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The key objective is to obtain fundamental information on neurobiological function and potential for rehabilitation for a brain system that is often affected in a host of brain disorders. The research will directly inform parallel studies with neurology and neurosurgery patients.

A retrospective assessment of these aims will be due by 16 December 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The societal burden of brain disease is increasing with over 12.5 million neurological events such as stroke in the UK per year, NHS England. Neurological events often affect the brain system for communication and thought (cognition), which is extensive. How this system works as network and how impact on it can be avoided or potentially rehabilitated is of urgent research need. However, much of this work cannot be conducted in humans and requires nonhuman primates because they have the closest similarity in this system to humans. The work in nonhuman primates will directly inform parallel research with human neurology and neurosurgery patients, including potentially informing surgical treatment approaches to ensure that during neurosurgical treatment the impact on this brain system is minimised to help to preserve thinking and communication abilities. The work will also inform a computational model of this brain system that is being built.

What outputs do you think you will see at the end of this project?

The work with nonhuman primates will provide the required information not possible to obtain in humans, showing how the different parts (nodes) of this brain network involving prefrontal cortex and the memory circuit functions. It will provide information on the key roles of the brain regions from the scale of neurons to populations of neurons. It will also provide information on neuronal information flow between regions. To emulate the impact on the system, we will use approaches such as deep brain stimulation, which is commonly used in neurosurgery patients for treatment, and non-invasive approaches like ultrasound that use sound waves to stimulate specific brain regions and as such carry tremendous potential for translation to humans. The work will also be used to build and test a computational model that requires but ultimately may lead to a reduction in the need for further invasive recording studies in nonhuman primates. The work will directly inform parallel studies conducted in patients that either have communication and memory impairments or are being neurosurgically treated. The outputs will include publications, technological advances, including computational modelling, and unique scientific resources made publically available. The proposed work will also create animal welfare information and insights, of relevance for advancing the 3Rs, including assessing refinement to optogenetic procedures using nanoligands for less invasive delivery of viral vectors. Other outputs are presentations at scientific meetings and public engagement via our outreach activities, social medial and media interest in the work.

Who or what will benefit from these outputs, and how?

The work will in the more immediate to shorter term generate scientific outputs and advance knowledge benefitting scientists and neuroscientific advances. During this project license timeframe or shortly after completion we also anticipate the work may assist with the development of techniques for humans, of direct relevance formedical teams treating brain disorders that may impact on the system. This could be in the form of scientific information that shows how best to avoid surgical impact on the system, for example, or

whether brain stimulation techniques could rehabilitate parts of the system and preserve thinking (cognitive) and communication behaviour. During this timeframe the computational modelling work could inform effort to model human cognition and brain function using artificial intelligence systems.

We also anticipate benefits during this timeframe on public interest in understanding how the brain works, what makes us unique or like other animals and what advances in diagnosis and treatment are being developed. In the short to mid range we anticipate being able to generate or encourage further advances in technology and resource sharing.

Longer term, in the 5-10 years after the PPL, we anticipate the work being able to inform clinical treatment or provide additional information needed for decision-making and neurosurgical planning or developing new treatment options. There could also be improved diagnostic approaches of patients, and there are new approaches in brain imaging techniques that are being developed to better stratify patients for treatment or provide differential diagnosis and better prognosis or recovery.

With the close collaboration with neurosurgery and neurology teams, we expect to be able to inspire and contribute towards concrete translationally relevant developments that in the longer run have the potential to benefit neurosurgery and neurology teams and their patients.

How will you look to maximise the outputs of this work?

One of the main ways in which we aim to maximise the outputs of this work are by the direct links to human studies, for example, the parallel studies in human neurosurgery and neurology patients using similar tests as in the NHPs and bridging neuroimaging and neurophysiology techniques that can be conducted in humans. This allows the insight from approaches that cannot be used in humans to better help to develop knowledge related to humans. Outputs will also involve animal welfare 3Rs advances presented at animal welfare meetings. For instance, we often present our work at the NC3Rs primate welfare meeting or other national and international animal welfare meetings.

Species and numbers of animals expected to be used

- Marmosets: minimally 4 and maximally 6 for each of the two objectives (2 and 5), total 8 or at maximum 12 marmosets

- Rhesus macaques: minimally 2 and maximally 3 for each of the six objectives, total 12 or at maximum 18 macaques

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult macaques are the ideal model for the human work on complex cognition because they uniquely can conduct the cognitive tasks and can provide the neurobiological information required, which is not possible in species more closely evolutionarily related to humans (such as apes) nor mammals more distantly evolutionarily related to humans. This

project can be achieved using minimally 12 rhesus macaques or maximally 18, over 5 years. Two of the six objectives require adult marmosets since they may be a better model system as justified in the PPL, but this needs to established. The marmoset work will require minimally 8 and maximally 12 marmosets to achieve those objectives.

Typically, what will be done to an animal used in your project?

The animals will be slowly acclimated to the laboratory environment and trained to perform one of the three cognitive tasks. They are motivated to perform the task by having controlled access to fluid (or food), which is provided to them for correct task performance. Fluid or food control is carefully regulated individually for each animal. The level of control is carefully chosen so that it just motivates the individual animal to complete the task without causing adverse effects on their health or wellbeing. The fluid/food control levels are regularly re-evaluated and adjusted. The expected adverse event with immobilisation is distress, which is minimized by slowly acclimating the animals to the procedures. The time of chair and head restraint is kept to a minimum (macaques: < 3 hours for brain imaging; < 5 hours for neurophysiological recordings; marmosets <3 hours). Non-invasive brain imaging is conducted with MRI or EEG under temporary head immobilisation. These brain imaging approaches are used to directly link the nonhuman primate findings to those in humans, to better translate the understanding of brain mechanisms to humans. In the animals, the functional MRI results will guide recordings from the key sites involved in the task in the frontal or temporal cortex during task performance. Access to brain neurons requires a surgically implanted recording chamber. Thus the animals will undergo an implant surgery once at the beginning of these procedures to implant any necessary chambers for recordings. This is important as it will allow us to show how neuronal circuits interact between different brain regions, which is not possible in humans. The use of deep brain stimulation, which is an important clinical treatment for human neurological disorders, will be compared with approaches that cannot be conducted in humans (activating or inactivating neurons with light or with neuroactive substances). This will allow us to causally implicate and evaluate the reversible manipulation of neurons in the key sites, which is unlikely to affect or be noticed by the animal. A potential adverse event is infection associated with the implant, which is minimised by always using aseptic techniques with anything involving the recording chamber and regularly using mild disinfecting agents on the implant and healing creams. We have also testing a new generation of MRI and more bio-compatible implants, and have innovated our use of MRI and X-rays to identify early any potential problems and when the do arise to guide veterinary treatment and to monitor treatment response. This approach has allowed us and our veterinary teams to successfully treat and respond to unexpected complications. At the end of the experiments the animals will be humanely killed during a non-recovery procedure and then the tissue studied, adding additional value to the research.

What are the expected impacts and/or adverse effects for the animals during your project?

Food or fluid control is conducted in a way to ensure that animals are not dehydrated nor show any adverse physiological or behavioural effects. The protocols are individually customised to the animals. If any issue with the animal typically involves lifting any restrictions until the issue is assessed and resolved. Our veterinary teams have not recognised or had to deal with any issues related to food or fluid control for any of our animals thus the impact is expected to be mild. Chair and head immobilisation can lead to discomfort and distress, which is monitored and addressed as soon as it is seen. To reduce the possibility of distress, immobilisation is conducted with positive reinforcement and each animal is acclimated slowly and at their own pace to avoid

distress. Surgical implantation of the head piece with recording chamber and craniotomy to conduct neurophysiological recordings is graded as having a moderate impact, which is minimised by appropriate post-operative care and innovation of a post-surgical cap that has at least anecdotally improved surgical outcomes. Infection is a possible adverse effect that is minimised with aseptic procedures involving the chamber and appropriate maintenance. Also MRI and X-ray monitoring of potential issues that might arise with implants is another innovation that is available and is being assessed as part of this project. Site-specific perturbation with deep brain stimulation or opto- or neuro-active substance only has a focal and reversible impact on neurons in the area that is typically not noticed by the animals, but can be quantified during its effect on their cognitive task performance. Terminal procedures under general anaesthesia are unclassified because the animal is anaesthetised and unconscious and is not allowed to recover after this final procedure.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All primate neuroscience protocols are as a precaution and in recognition of the sensitive work being done with these special species is graded as Severe by the Home Office. However, the project license and end points aim to ensure that no animal suffers severely with humane endpoints appropriately defined. Also rigorous regular retrospective assessment with our NVS, veterinary teams and NACWO annually returns the actual severity experienced by animals completing this work and for the last two project license periods has been no more than moderate for our animals. Moreover given the new refinements that are being assessed we expect that going forward that nearly all (100%) of the animals will stay within the moderate severity category. This may not always be possible but is certainly our goal with the humane endpoints defined accordingly.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 16 December 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

LIMITATION OF TECHNIQUES AVAILABLE FOR HUMAN STUDY

Functional Magnetic Resonance Imaging (MRI) shows where the brain uses more oxygen (is more ‘active’) but not how neurons in these brain regions are working. Other non-invasive approaches commonly used in humans also have limitations. Patient lesions are often too broad or variable between patients to relate to specific cognitive abilities. Although in some cases neuronal recordings can be made in patients being evaluated for epilepsy, the placement of electrodes is clinically rather than scientifically guided, the tissue being evaluated is potentially pathological, and causal relationships involving brain manipulation are not possible. We conduct this type of human work, but also recognise its limitations and where animal research can address them. Studies in the suitable animal models can provide the required level of understanding of neural mechanisms, circuits and casual influences not possible with humans, however until recently which species can model these aspects of human thinking was not clear.

Primates like macaques are the ideal model system for this proposal because they are the species that are most closely evolutionarily related to humans in which neurobiological data can directly inform our work with neurosurgery and neurology patients. More distantly related species such as rodents cannot learn the cognitive tasks that are required. Macaques also have a neurobiological system that is closest to humans particularly for the brain system for thinking under study here.

Marmosets are required for a more limited role on this project licence. For the question on how the brain combines information from the different senses (audition/vision), which is called 'sensory convergence', they may be a better model system than macaques because of their larger social groups and more group based infant rearing approach throughout their life course, both of which are guided by their audio-visual communication abilities. They are also preferred for use for questions that can also be addressed in macaques because they are more distantly evolutionarily related from macaques and humans, as potentially less sentient primates. Finally, marmosets are an important animal model for the pharmaceutical and scientific communities, helping to establish which information can translate from rodents to primates more closely evolutionarily related to humans. Thus a more limited role for marmosets is justified on this proposal.

Which non-animal alternatives did you consider for use in this project?

Alternatives that we use whenever possible: It is important to note that we conduct work humans whenever it can address the scientific questions. Our group runs an active programme using human MRI, and we rely on human including patient work to answer scientific questions wherever possible. In this case we do not conduct nonhuman animal research (Replacement). Examples of this include any questions where we are interested in which brain regions are involved (‘activated’), but are not concerned with the basis for or the neuronal mechanisms of the activation patterns. We also use computational modelling of behavioural results whenever possible which allows us to make predictions and assess the quality of the datasets.

Why were they not suitable?

The non-invasive or minimally invasive nature of the human work means that such experiments are highly limited for understanding how neuronal networks work at the fundamental level of individual and populations of neurons. Neuronal recordings in neurosurgery patients are not a surrogate for the work in animal models and the tissue being monitored in these patients for surgical removal is pathological. Furthermore, studies of thinking cannot be simulated, conducted in vitro in slice preparations, or conducted in anaesthetised animals.

For the work that requires macaques, we often first use non-invasive imaging such as functional MRI and EEG to more directly link and relate the human and nonhuman primate work. Then we use the most efficient approaches to study neuronal mechanisms in the fMRI-identified brain network (Refinement), and use the smallest number of animals (Reduction). Our work is 3Rs relevant and strives to advance both scientific discovery and animal welfare, wherever possible. This project will thus also feature several 3Rs related developments.

A retrospective assessment of replacement will be due by 16 December 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We abide by the revised ARRIVE 2019 and the PREPARE guidelines, which provide specific information to standardise the reporting of animal research and what information needs to be reported so that other research groups can fully understand things like the experimental methods and the data produced. In preparing the project licence have consulted the PREPARE guidelines as well as the NC3Rs Experimental Design Assistant to ensure robust experimental design, to develop the planned analyses and to assess the sample sizes needed. This was also informed by our prior published effect sizes described in detail in the project license. We thus aim to use the fewest animal numbers possible to generate peer-reviewed publishable results that are robust in each animal and where effects replicate across the individuals and are likely to replicated in larger samples of animals.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

I regularly consult with computational scientists on analysis and experimental design in the UK and US. I have also taken guidance from the NC3Rs Experimental Design Assistant on the statistical design needed to ensure, in particular, efficiency in design for the fewest animal numbers.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

These numbers based on sample size calculations are a starting point but there is uncertainty in whether 2 or 3 animals will be sufficient, which depends on the replicability and statistical robustness of the results. An additional macaque per project might be

needed but this won't be known until the results from the first two animals are known. We recognise that behavioural neuroscience labs working with nonhuman primates are very limited in the numbers of animals that they can put forward for any given publication. The Human Connectome Project is a good example of the international community coming together to be able to provide thousands of connectome-based neuroimaging datasets. Thus I am involved in efforts to establish a primate Connectome Project equivalent, which would mean that my lab will contribute data along with other labs for maximal effect. So far we have contributed structural and resting state data in as many of our animals as we can. The way we collect these data could make no animal welfare difference but provide a more systematic way in which the community can achieve the large sample sizes to support insights by the community that are only possible via international coalitions.

A retrospective assessment of reduction will be due by 16 December 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

HOW ANIMAL SUFFERING WILL BE MINIMISED

Since there is considerable investment in forming a good working relationship with these individuals, who will by the first study be very well adjusted to the MRI scanner and the laboratory environment, the work can only be advanced by well-adjusted animals that are motivated to correctly complete the tasks and the research objectives. Since the approaches are longer term, this also requires that the animals stay healthy and that we minimise evident suffering or distress. We have had good success in acclimating the animals to the procedures and these animals are contributing toward understanding and improving brain function in animals (relevant for veterinary medicine) and in humans (relevant for medical science). Some initial stages of behavioural, MRI and electrophysiology sessions are of moderate severity but in many cases the animals acclimate to the procedures. For example, the animals acclimate to the confined space in the MRI scanner by training in our 'mock scanner' training setup. The procedure severity drops with proper acclimation, but we aim to measure this more objectively using multi-parameter measurements. We also continuously monitor the animals for distress, take steps to avoid it in all the procedures, and have established humane endpoints for animals proceeding to terminal procedures.

Why can't you use animals that are less sentient?

The work requires macaques and marmosets and cannot be completed with less sentient animals because only these animals can conduct the cognitive tasks as do humans. Anaesthetised animals cannot be studied because behaving animal work is required. This work is not currently thought to be possible in any other species. It cannot be conducted in species more closely evolutionarily related to humans (apes) because neurobiological work in apes is not allowed in the UK. Thus macaques are the ideal model system and marmoset work will be used to a more limited extent as required as for the sensory convergence components where they may be a better model, although that needs to be established.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We continuously strive to refine our approaches. As examples, we have been using training strategies with the animals that had been developed to prepare children and elderly individuals for MRI scanning. This involves giving the individual practice in a pretend or "mock scanner" so that they get used to the confined space in the scanner and to the noise that the scanner generates. The implant techniques used have been, and continue to be, improved to cause minimal interference with the animal's normal activity and to remain functional for a prolonged period. Several 3Rs related projects will be conducted in parallel to the scientific objectives on this programme of work. For example non-invasive head immobilisation options can be used to delay surgical implants in many of the animals on study and participating in MRI or behavioural experiments only. A surgical implant is required for electrophysiological recordings to accommodate a recording chamber, but even in this case the noninvasive head immobilisation might allow for more compact implants and we are assessing and refining MRI-compatible implants as part of this project license. A non-invasive head immobilisation option has now become a key 3Rs related project for testing in Stage 2 of the current project license and will continue to be evaluated. Likewise, there is a strong need to evaluate the physiological, behavioural and scientific impact of different approaches for motivation, as a number of best practice recommendations require an evidence base to evaluate whether the recommendations being made have a measurable positive impact on animal welfare and do not have knockdown effects on the 3Rs (e.g., increasing animal numbers for selection that results in little to no scientific advance). Finally, we aim to adopt and help to refine methods for measuring multi-dimensional parameters that can be used to evaluate any cumulative effects by the neuroscientific procedures. These objectives will obtain important data to evaluate different procedures for refinement of current procedures.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We abide by the revised ARRIVE (2019) and PREPARE principles and follow the NC3Rs Guidance on Primate Welfare and Housing for Macaques and Marmosets. The ARRIVE and PREPARE guidelines help to standardise the reporting of studies with research animals so that other research groups can fully understand things like the experimental methods and the data produced. We also always rely on the latest publications in macaques and marmosets to stay on top of refinements in both of these species.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We regularly attend annual meetings that report on the latest animal welfare advances. As well as advancing scientific and biomedical knowledge, our prior project licence contains

several 3Rs relevant aims. This type of work has previously led to development of a non-invasive head immobilisation and another on using MRI and x-ray to assess implant stability and to help to diagnose potential complications often before they have a behavioural impact on the animals. Such efforts are motivated from refinement needs that we've seen as part of scientific meetings and the annual NC3Rs primate welfare meeting. This project licence will also include several new 3Rs aims that will run in parallel with the scientific objectives and thus not require further animals.

A retrospective assessment of refinement will be due by 16 December 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

40. Protection after heart attack or stroke

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Heart attack, Stroke, Therapy

Animal types	Life stages
Mice	adult, pregnant, juvenile, neonate, embryo
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand how heart and brain tissue can be saved from damage following a heart attack or stroke.

A retrospective assessment of these aims will be due by 18 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these

could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Heart attack and stroke are the leading cause of death worldwide and present a huge burden on health systems such as the NHS. These diseases occur when an artery supplying either the heart muscle or the brain respectively is blocked, disrupting the delivery oxygen and nutrients as well as the removal of waste products. This inevitably leads to death of the tissue that is without blood supply, which can lead to both severe long-term disability and increased mortality in patients.

What outputs do you think you will see at the end of this project?

This project will develop and test novel protective drugs for heart attack and stroke, as well as the resulting organ damage of chronic heart failure or chronic neurological disability, in order to pave the way towards the use of these compounds in patients. The information gathered from this project will allow us to see how these substances affect the heart or brain and what doses and treatment regimens are most likely to be effective. We will also examine whether lifestyle changes (such as diet and exercise) or pre-existing diseases alter their effectiveness.

Treatment of an acute heart attack and stroke has remarkable improved over the last decades resulting in fortunately low acute in-hospital mortality. However, once patients survive the acute event, they are prone to chronic changes of the organs, resulting in chronic heart failure or chronic neurological disability. The present project will therefore address both of these important endpoints: the acute damage to the heart or brain, and, maybe even more important, chronic changes leading to heart failure or disability.

We aim to publish in high-ranking journals in order to inform the scientific community. I also regularly take part in public engagement activities, such as Pint of Science, University-wide Science festival, "Naked Scientist" and appear in the popular press in order to inform the general public about the progress and new findings of our research. Ultimately, the above results and achievements will pave the way towards the use of novel compounds in patients with heart attack, heart failure, and stroke.

Who or what will benefit from these outputs, and how?

Initially, our fellow scientists will benefit from our findings about the underlying mechanisms of health and disease in heart attack and stroke. We publish in prestigious journals and are active members of many scientific societies with regular participation at meetings.

In the second line, we anticipate bringing at least one of our novel compounds towards use in patients with heart attack or stroke within the next 5 years. Drug development is a long and complicated process, but we are confident that this project will enable us to move forward to a first-in-man study on this timescale.

How will you look to maximise the outputs of this work?

My group is very well connected, not only locally, but also on a national and international level. The quality of our animal work has made my group one of the leading groups in the

country investigating damage during heart attack. We regularly publish our results, including negative outcomes or unsuccessful approaches.

My group and my collaborators are very active on various outreach channels, including twitter, popular press, radio, and websites. The general public has access to all our findings and we widely share our knowledge. We have a large number of collaborators world-wide, which work together with us to maximise our findings and make translation towards patient care more likely.

Species and numbers of animals expected to be used

- Mice: 4400
- Rats: 800

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Since the effects on hemodynamic parameters and the elimination/distribution of compounds are essential parameters of a pharmacotherapy, isolated organ systems cannot be used. It is not possible to gain this information in vitro since the whole-body system needs to be intact due to the influences that administration, distribution, metabolism and excretion have on the availability and efficacy of the compound. Furthermore, changes in exercise levels, diet or pre-existing diseases cannot be mimicked without animals.

Rodents are the smallest possible species where interventions, such as ligation of a coronary artery or occlusion of a brain artery in order to induce a heart attack or stroke respectively are possible.

In addition, rodents offer the possibility to use genetically-altered models in order to mirror patient conditions (such as pre-existing diseases) more closely.

Typically, what will be done to an animal used in your project?

Typically either purpose-bred genetically-altered animals are used or genetically normal mice will be used. The breeding protocols follow standard breeding procedures.
In a subset of animals certain conditions such as exercise, administration of substances or alteration of diet will be applied in order to mirror more closely the conditions in humans. This includes pre-existing lifestyle (exercise or diet) or pre-existing medication. In addition, if the animals are on the voluntary wheel running protocol, they will be single housed. Finally, animals will be subjected to surgical procedures to induce either a heart attack or a stroke. These final procedures will be performed either under terminal anaesthesia and the animal will not be allowed to recover, or animals will be allowed to recover. After a certain recovery period, the animals might develop heart failure or neurological disability which mirrors very closely the conditions of patients surviving a heart attack or stroke following treatment.

What are the expected impacts and/or adverse effects for the animals during your project?

Overall, the animals will only suffer mild discomfort and no lasting harm. Most of the interventions, such as exercise or dietary intervention, are not expected to have any discomfort and certainly no harm to the animals. Rarely, very mild discomfort will occur. The surgical procedures will be performed solely under confirmed general anaesthesia and in case of the recovery protocol, adequate pain medication will be given during the wound healing and recovery period from surgery. The development of heart failure or neurological disability itself is without any pain. However, the animal might experience moderate suffering due to the development of heart failure, such as fatigue and breathlessness, or in case of stroke, neurological disabilities.

Nevertheless, some animals may die during surgery due to complications in the procedure itself or the anaesthesia. These deaths will be rare (<5%) and will happen during adaequate full anaesthesia. In addition, very rarely (<3%) animals will die during the follow-up period post surgery. These deaths are almost always due to fatal cardiac arrhythmias which are entirely pain-free and can be compared to "sudden cardiac death" in patients with heart conditions. Due to the sudden and unexpected nature of these deaths, there is no possibility to make any prevention beforehand.

We further aim to perform interventions, such as imaging or administration of substances. Only rarely these interventions cause mild discomfort.

Taken together, the likely adverse effects will not exceed a moderate level at any point.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Non-recovery: Mice 40%
Rats 100%
Mild: Mice 30%
Moderate: Mice 30% (of which <5% are expected to die)

What will happen to animals at the end of this project?

- Kept alive

A retrospective assessment of these predicted harms will be due by 18 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Information about the distribution of the drugs, effects on the heart and brain, or blood pressure and exercise tolerance cannot be answered using cell-based models.
The novel drugs which will be tested with this license have already undergone extensive testing in suitable non-regulated models in cell culture, isolated cells or isolated organ preparations. Therefore, we already know most of the mechanisms of action and have information about possible toxicity, dosing and the most effective treatment regimen. However we need to confirm efficacy in animal models before we can translate these compounds to human use. Furthermore, these animal models have to mimic the situation of patients as close as possible. This can be done by an alteration of diet, exercise, application of pre-existing medication, and the use of pre-diseased genetically-altered models.

Which non-animal alternatives did you consider for use in this project?

We are already using isolated organ applications for testing in the heart (Langendorff preparation). This cannot be done with the brain.

Why were they not suitable?

Cell lines cannot mimic important conditions, such as exercise, and do not have crucial confounding elements, such as circulation or the blood brain barrier.

There have been decades of research trying to find a suitable cell-based model of ischaemia (lack of oxygen to tissue)/reperfusion (re-introduction of oxygen and blood flow to tissue), but so far none have been found.

A retrospective assessment of replacement will be due by 18 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals is determined by previous experience with an identical methodological approach under a different license. Furthermore, we will use power calculations based on our previous data to calculate numbers for each individual experimental setting.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

I use the NC3R Experimental Design Assistant (EDA) in order to thoroughly design the studies.

In addition, I am on an EU committee (COST Action "Cardioprotection") which aims to standardise ischaemia/reperfusion experiments throughout Europe considering the 3Rs as well as highest scientific standards. Within this consortium, my group is one of the reference groups for acute heart attack in mice.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Wherever possible, we try to assess as many parameters in a single animal as possible and reduce the numbers in one treatment group to an absolute minimum that the statistical analysis will allow us as determined by power analysis. This includes performing sophisticated imaging techniques which allow us to gain much additional information about the heart attack or stroke in a single animal.

A retrospective assessment of reduction will be due by 18 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The present project will use either wild-type mice and rats or genetically-altered mice. The geneticallyaltered strains are lacking important mechanistic elements which helps us to understand the mechanisms of how we can protect against heart attack or stroke. We are already breeding these genetically-altered lines under a different license and the animals do not show any clinical signs.

The main aim of the license is to test protective effects in heart attack and stroke as well as during the following recovery period where the animal might develop heart failure or neurological disabilities. Both surgical models will be performed under adequate anaesthesia and pain medication during the immediate recovery. While long-term recovery is pain free, it might however cause moderate harm due to signs of heart failure (such as breathlessness and fatigue) or neurological disability (such as inability to move and climb). Since the experiments are aimed to find treatments to ameliorate these long-term effects,

there are no models available with less suffering, The chosen models to examine long-term effects after heart attack or stroke are the ones with the least suffering for the data obtained. Many other models used in the literature are of increased harm.

The alteration of diet or exercise and administration of substances are the most refined models used to determine these important factors which can influence the outcome of heart attack and stroke. There will not be any lasting harm and only mild discomfort for the animals due to these models.

Why can't you use animals that are less sentient?

Mice and rats are the most useful species from which it is possible to obtain relevant and meaningful physiological and pathophysiological information. Especially non-invasive imaging such as MRI and PET cannot be performed in smaller species. Furthermore, mice allow genetic manipulations in order to more specifically study the underlying mechanisms.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We closely monitor the animals throughout the experiments. This includes the use of sophisticated heart and brain function monitors, such as Ultrasound, ECG and blood flow monitor (doppler), as well as temperature control. If anaesthesia is applied, the efficacy is monitored closely throughout the procedure. Should any anaesthesia or surgical problem occur which would potentially harm the animal, the experiment will be terminated and the animal humanely killed.

In protocols or procedures where anaesthesia is not used, animals will be monitored for any deviation from normal health and behaviour and should any signs appear animals will be killed immediately.

The animals are allowed to acclimatise to either the exercise protocol or the water supplementation and dietary changes. In protocols where treadmill running is used, animals have warm-up acclimatization prior to the exercise protocol.

The animals are allowed to acclimatise to either the exercise protocol or the dietary changes.

Within the previous license, we gained much experience in pain management after the described surgical procedures. While the development of either heart failure or neurological disability itself is painless, we will make sure that adaequate pain medication is given after surgery.

In addition, the animal will be kept in a warm environment for the immediate recovery and receive mashed food as long as necessary.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the NC3Rs Experimental Design Assistant (EDA) see https://www.nc3rs.org.uk/experimental-design as well as the following guidelines: for pilot studies (https://www.nc3rs.org.uk/conducting-pilot-study); ARRIVE guidelines, https://www.nc3rs.org.uk/arrive-guidelines; IMPROVE guidelines for stroke models: https://www.nc3rs.org.uk/news/improve-ing-animalwelfareexperimental-stroke-research;

published guidelines to assist with planning animal research and testing, such as the PREPARE guidelines: http://journals.sagepub.com/doi/full/10.1177/0023677217724823

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I receive regular updates via the Biological Service of the University of Cambridge about news and advances in the 3Rs.

A retrospective assessment of refinement will be due by 18 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

41. Provision of Biological Materials

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Positive, Control, material, Diseases, Endoparasites

Animal types	Life stages
Cattle	adult
Sheep	adult, juvenile
Goats	adult
Pigs	adult
Horses, Donkeys, Ponies, Cross-breeds	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This project is to provide a service for the supply of blood and other biological materials for use as controls in diagnostic testing, quality assurance, and research projects requiring biological materials. The samples supplied from the various species will be used in evaluating diagnostic tests for a variety of animal diseases. The project is demand driven and all individual requests for samples will be ethically approved and only supplied once a written case outlining why the samples are required and why no alternative source is possible. The quality of samples produced and their timeliness are important to the success of the research that this project licence supports.

A retrospective assessment of these aims will be due by 14 July 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

There is a requirement to supply blood, faeces, helminth eggs and other materials for scientific purposes, for use as standards and negative control material, in the development of and the maintenance of laboratory techniques, in undertaking research projects, at this and other establishments.

Monthly supply of cattle blood to for a Haematology QAU scheme. This is part of an independent, accredited proficiency testing service for veterinary diagnostic laboratories both nationally and internationally.

Supply of blood products to research projects at the establishment and research projects carried out by collaborating institutes. Pig blood samples are mostly taken for use in in-vitro studies using different cell types involved in the provision of vaccinal immunity against various porcine viral diseases. Ruminants were also sampled for the same reason, the main disease of interest being bovine TB.

The Equine Encephalomyelitis project is aimed at developing a test to detect the Eastern (EEE), Venezuelan (VEE) and Western (WEE) encephalitides viruses. The UK is currently free from EEE, VEE and WEE, but these viruses are endemic in several US locations. More recent reports indicate the increased regularity of EEE, with human fatalities.

Therefore, there is a more urgent need for a welldeveloped and robust test to detect these viruses for several reasons. Based on the World Organisation for Animal Health manual and an existing standard operating procedure for Equine Encephalomyelitis, the EU Exit team is aiming to develop the EEE/VEE/WEE haemagglutination inhibition test. This test specifically lists geese red blood cells as part of the test ingredients due to the sensitivity of this type of cells.

Helminth eggs collected for External Quality Assurance (EQA) exercises, for our organisations independent, accredited, proficiency testing (PT) service provided by our Quality Assurance Unit (QAU).

What outputs do you think you will see at the end of this project?

This is a service licence supplying biological materials to both diagnostic tests and research, so the outputs are volumes of blood and other biological control material.

Who or what will benefit from these outputs, and how?

The supply of this blood and biological material allows peer reviewed research to be undertaken at the organisation and other public health organisations, with the associated increase in scientific publications and outputs. These benefits are more longer term (months to years depending on nature of the disease and the research project) due to cumulative nature of scientific research in working towards a better understanding of disease processes and the ability to control them with the development of new tests and vaccines.

Supporting the ongoing testing requirements from the establishments customers, for use of complement fixation tests or similar tests for the purpose of international trade and diagnostic testing.

The quality assurance unit will be able to continue to supply proficiency testing material to laboratories fulfilling the needs of the establishment and other laboratories.

The diagnostic tests it supplies underpins the national capacity for disease diagnosis with the associated impact on animal welfare, also its import/export testing which is necessary to prevent the spread of certain diseases. The benefits of being able to run these tests are realised mostly short-term.

How will you look to maximise the outputs of this work?

The organisation made a strategic decision some years ago that all supply of negative control blood and biological material required within the organisation should be under one service PPL to maximise the 3R's benefit by adding better oversight over tissue requests and reducing the number of animals needed through re-use. Whenever possible sample requests from different users will be aligned to that one sample event provides material for several projects, thus reducing the sample frequency for each animal. This benefit has been further maximised by supplying control material to other institutes.

The helminth eggs are provided to an EQA scheme, which is recognised by national accreditation bodies, it is ISO/IEC 17043 accredited.

Species and numbers of animals expected to be used

- Cattle: 510
- Sheep: 63 Goats: 10
- Pigs: 40
- Domestic fowl: No answer provided : 10

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The animals used on this licence are the main commercial livestock, equine and poultry species of disease control interest in the UK and the materials supplied with this licence will be used for diagnostic, import/export or laboratory tests (including research) for disease these species. For test performance (sensitivity/ specificity it is necessary to use

the same species. The vast majority of animals used will be adult, on occasion juveniles may be used if required.

For the supply of helminth eggs lambs will be used as they are the main domestic species susceptible to the strain of helminth used, they have to be juvenile as adult animals develop a resistance to infection.

Typically, what will be done to an animal used in your project?

For Protocol 1

Withdrawal of blood by venepuncture from a superficial blood vessel. In pigs this could also include the jugular or anterior vena cava. (AA/AB local)
Optional Manual withdrawal of faeces from the rectum and/or rectal swabbing (mammals only).

Optional Nasal swabbing mammals only. (AA)

Steps 1, 2 and/or 3 may be repeated in situations where serial blood samples are required from the same animals. Frequency will be limited to 10 times a month in these situations and up to 30 times a year. (AA/AB local)

Optional chickens and turkeys only, euthanasia by exsanguination by cardiac puncture under deep terminal anaesthesia (AC).

For Protocol 2

Oral dosing with helminth eggs, then collection of faeces once the helminth are established in the gut using a bag which is held in place using a harness.

What are the expected impacts and/or adverse effects for the animals during your project?

Protocol 1

Pain caused by the insertion of a hypodermic needle according to good veterinary practice, during the sampling. Stressed caused by restraining the animals during procedures.

Sterile blood sampling procedures and careful handling of the needles plus good restraint will be used to minimise damage to the blood vessels and inflammation caused to the vein.

Heamatomas will be prevented or controlled by pressure on the site immediately on removal of the needle/cannula.

Blood withdrawn from animals will be kept to the minimum practicable volume, but will be governed by the following:

No more than 15% of the blood volume should be removed in any 28 day period.
No more than 10% of the animals' blood should be taken in any 24 hour period.
Total blood volume will be estimated as 60ml/kg body weight.

Protocol 2

Mild discomfort during single oral dosing.

The acclimatisation to the faecal collection harness and the associated handling by competent staff during collection and change of the harness is well tolerated and subthreshold.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild

What will happen to animals at the end of this project?

- Kept alive
- Rehomed
- Killed

A retrospective assessment of these predicted harms will be due by 14 July 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Biological material such as blood and faeces are the basis for a lot of laboratory tests involved in animal health and veterinary diagnosis, therefore the negative control material has to replicate this.

The strain of helminths required are obligate parasites.

Which non-animal alternatives did you consider for use in this project?

This licence supplies biological material to tests that are either already established for disease control purposes or in the process of being developed.
Wherever possible samples collected post mortem will be used to supply the need for material, however as these laboratory tests are used to diagnose disease in the live animal, there is often the requirement to use fresh material, to avoid post-mortem changes issues.

Why were they not suitable?

These tests require ante-mortem samples and there is currently no alternative. There is no artificial way to replicate the gut for generation of helminth eggs.

A retrospective assessment of replacement will be due by 14 July 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

By reviewing the predecessor of this licence, the requests for samples using the previous licence and the animals used throughout its duration.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The experimental design is based on matching demand for blood through the request form to numbers of animals maintained to supply the blood. Animals will also be sampled on a rotational basis in order to reduce the burden to each individual.

The helminth egg production has to occur every year as the helminth eggs deteriorate once outside the body, so have to be refreshed annually.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The number of animals used is minimised by re-using them. This allows a smaller number of animals to provide control blood across the establishment and other research establishments. Whenever possible, tissue requests from different users will be aligned to reduce the number of sampling events for each animal.

For helminth production two lambs are used to provide company for each other (essential for lambs as flock animals) and minimise the numbers of animals used each year.

A retrospective assessment of reduction will be due by 14 July 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Withdrawal of blood by venepuncture from a superficial blood vessel. In pigs this could also include the jugular or anterior vena cava.

Optional Manual withdrawal of faeces from the rectum and/or rectal swabbing (mammals only).

Optional Nasal swabbing (mammals only).

Steps 1,2 and/or 3 may be repeated in situations. Sampling frequency will be limited to 10 times a month in these situations and up to 30 times a year.

Optional chickens and turkeys only, euthanasia by exsanguination by cardiac puncture under deep terminal anaesthesia.

Faecal sampling from the animal's environment where possible.

For helminth production the use of two lambs minimises the infection/collection period.

Why can’t you use animals that are less sentient?

The samples request state mature life stages. For the number of small blood samples required this would lead to an unacceptable level of animal usage if these were done under terminal anaesthesia. The helminth required is an obligate parasite of sheep.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The establishment has a group of experienced and knowledgeable staff who are able to perform the necessary procedures and restraint quickly and efficiently. Personal licence holders and animal technicians undergo regular refresher training as per the establishments competency framework.

Where possible the animals will be acclimatised to the restraints and handling techniques for the procedures they will undergo.

Positive reinforcement will be used as part of the acclimatisation and training process.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Home Office The Harm–Benefit Analysis Process _Advice note
Home Office Guidance to Animal (Scientific Procedures) Act 1986
Home Office Code of Practice for the housing and care of animals bred, supplied or used for scientific purposes
OIE (World Organisation for Animal Health) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

By attending regular Named Animal Care and Welfare Officer meetings and through on going communications with the NTCO and NIO at the establishment as well as reviewing literature, staying informed of recommended refinements/replacements, engaging with stakeholders such as National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), Laboratory Animal Science Association (LASA) and RSPCA.

A retrospective assessment of refinement will be due by 14 July 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

42. Regulatory Assessment of Chemical Safety for Aquatic Vertebrates (Fish)

Project duration

5 years 0 months

Project purpose

None selected

Key words

Fish, Environment, Chemical, Risk

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The aim of this project is to generate high quality data for regulatory risk assessments of chemicals that have the potential to reach the aquatic environment.

Data for regulatory risk assessment is a necessity under many national jurisdictions. It is used to ensure adequate protection of wild fish and other aquatic organisms from chemicals that are released into the environment by the activities of man.

A retrospective assessment of these aims will be due by 12 September 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

Regulatory risk assessments are made to help with decisions for the proportionate regulation and management of chemicals that could move into aquatic environments. The

benefits of the regulatory risk assessment process are twofold: • Chemical products that are a demonstrable risk to fish and the aquatic environment are ruled out of marketing and manufacture. • Chemical products that are demonstrably safe for the aquatic environment are authorised for use, which in turn accrues all of the benefits intended from the development of that product, be it improved protection of crops, or animal or human health.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

The species of fish selected will depend on the purpose of the test and the regulatory context. For example, the majority of initial testing for EU regulations will use a representative cold water species, namely Rainbow Trout. Additional testing for further characterisation of risk tends to use smaller, fast growing species, namely Fathead Minnows, Zebra Fish and Rice Fish. Assessments of Bioaccumulation in Fish can use larger species, namely Common Carp and Bluegill. In total, up to 11,000 fish may be used over a period of 5 years, to generate regulatory data on this project.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

The strategy for testing is carefully managed to ensure that all existing data on the properties and toxicity of the chemicals is used in order that only the minimum testing needed is carried out. However to establish the risk of the chemical to fish, testing in the initial stages of risk assessment is classified as severe. The fewest possible numbers of fish are used, but the fish are not fed, and some of those fish are likely to die. To avoid this as far as possible, the test is short (maximum of 4 days) and the fish are observed very frequently. Where signs of impending death are seen, humane interventions are taken. This involves removing affected fish and humanely killing them immediately. All testing is performed according to the International Test Guideline (OECD 203 Fish; Acute Toxicity Test).

Some testing is designed to assess the survival and growth of very young fish fry. The young fish larvae are observed frequently while they grow up over several weeks. They are cared for and fed in the usual way. Where signs of impending death are seen, humane interventions are taken. This involves removing affected fish and humanely killing them immediately. All testing is performed according to the International Test Guideline (OECD 210; Fish, Early Life Stage Toxicity Test).

In the later stages of risk assessment, the affects on the fish are less severe, with the outcomes being effects on growth rate in juvenile fish. It is necessary to anaesthetise fish for short periods so that they can be accurately weighed. These growth measures take several weeks to determine and the fish are cared for and fed in the usual way. This test is

considered as moderate severity. Where fish do not eat normally and become thin, humane interventions are taken. This involves removing affected fish and humanely killing them immediately. All testing is performed according to the International Test Guideline (OECD 215; Fish, Juvenile Growth Test).

Some parts of the risk assessment process look at how fish accumulate the chemicals that they are exposed to, and how they leave the system of the fish after exposure. During this process, fish are cared for and fed in the usual way. This test is considered as mild severity. Where fish do not eat normally and become thin, humane interventions are taken. This involves removing affected fish and humanely killing them immediately. All testing is performed according to the International Test Guideline (OECD 305; Bioaccumulation in Fish, Aqueous and Dietary Exposure).

The European Union has also recently required that many chemicals are assessed for their ability to act as endocrine disruptors. In fish, this assessment involves breeding fish in defined groups, and assessing the number of eggs they can produce when exposed to the chemical. At the end of the test, fish are terminally anaesthetised and sampled for circulating levels of egg protein hormones, which when elevated in males, indicate that endocrine disruption is occurring. This test is considered as moderate severity. Where fish do not breed normally and become bloated with unlaid eggs, humane interventions are taken. This involves removing affected fish and humanely killing them immediately. All testing is performed according to the International Test Guideline (OECD 229; Fish Short Term Reproduction Assay).

All the fish used will be humanely killed at the end of each test, and no fish is used for more than one test. The majority of testing is performed for EU regulatory requirements. In some cases, tests may be performed for other worldwide jurisdictions, and may use tests with exact equivalency to the OECD Test Guidelines, but such test are only undertaken with the express permission of the Secretary of State.

A retrospective assessment of these predicted harms will be due by 12 September 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

Tests using fish are currently required by regulators and are considered the only way to be sufficiently certain that wild fish will be protected by the results of the risk assessment. However, research is on- going to look for alternatives ways of being equally sure of protecting wild fish without testing fish in laboratories (e.g. by using living cells cultured from fish gills). Currently no alternative approach is accepted by the regulator, however on every occasion over the 5 year life of the project, we will ensure that:

The testing is required to satisfy a regulatory imperative.

Satisfactory data is not already available.

No non-animal alternative method would be acceptable to the regulator.

A retrospective assessment of replacement will be due by 12 September 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

It will always be established that any testing has a clear regulatory requirement for the protection of wild fish in the aquatic environment, and that unnecessary repeated testing does not occur, by ensuring that adequate, acceptable data for fish does not already exist.

The minimum number of fish that can be used is determined by the regulatory guideline that ensures the scientific robustness and credibility of the statistics used to determine the result of the risk assessment.

Some guidelines allow for a further reduction in fish numbers in certain circumstances, e.g. when the chemical has properties that allow prediction of how it will interact with tissues reliably, and testing can be minimal to check these models. These minimised testing approached will be used as an alternative wherever is it possible to do so.

A retrospective assessment of reduction will be due by 12 September 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The species used are those recommended by regulatory guidelines. Sometimes there is a choice, and in these cases, the species that is the most representative of the wild fish in the environment to be protected are chosen. In some cases, the fish used are not directly comparable with the population to be protected, but instead are fast growing which means that the length of the tests can be shorter.

All of the species used prefer to live in groups, and so no fish will be deliberately isolated. Where a fish is isolated by circumstances, this will not be allowed to continue for more than a few days.

Fish are also stressed by disturbance and handling and so these will be kept to a minimum, and where handling is necessary, it will use ways that are known not to cause any damage to gills or scales.

In some tests, it is likely that a proportion of the fish may die. Where this is the case, the fish will be observed very frequently and where signs of impending death are seen, humane interventions will be taken immediately. This involves removing affected fish and humanely killing them. Additionally, any fish that is showing other signs of sickness beyond that expected will also be humanely killed straight away. For example, fish may not grow as they normally would, or may not breed as they normally would, and this would be expected in some cases, but any fish that is visibly losing condition, or is visibly egg-bound, would be humanely killed.

A retrospective assessment of refinement will be due by 12 September 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

43. Safety evaluation of products to support the health and welfare of farmed fish

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)
- Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

No answer provided

Animal types	Life stages
Atlantic salmon (Salmo salar)	juvenile, embryo, neonate, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

This project aims to support the safety evaluation of new products, including veterinary vaccines, therapeutants, feed materials, feed additives and biocides, intended for use in the production of farmed fish. The principal objectives are (a) to provide data on the safety of the candidate products in fish, (b) to characterise metabolic pathways, distribution in the major organs and changes in tissue concentrations of the products and (c) to describe the biological responses of the fish to the product at cell and tissue level.

A retrospective assessment of these aims will be due by 01 October 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

These studies are necessary (i) to enable assessments of the safety of the products in the fish species for which the products are intended (the target species), including regulatory assessment for the purposes of product licensing, (ii) to identify the preferred treatment regime and to permit assessment of tissue concentrations during and following treatment, including regulatory assessment to determine withdrawal periods for the protection of consumers, and (iii) to understand the mode of action at cell and tissue level so that the products can be used safely and effectively.

What outputs do you think you will see at the end of this project?

The expected benefits of this project will be to:

Establish a platform which clients can access to develop products for aquaculture. A shortage of capacity at present is restricting the availability of new products for farmed fish.

Support the evaluation, development and licensing of new products to improve the health and welfare of farmed fish. These products will be used by salmon farmers to reduce losses due to salmon lice, gill disease and other infectious disease.

Generate high quality data to ensure that new licensed products are demonstrably safe for the animal, consumers and the environment. These data will be produced to the internationally recognised quality standards required by relevant regulatory authorities.

Support the production of fish as food that is safe, healthy and nutritious, economically sustainable, environmentally acceptable and produced to the highest animal welfare standards.

Tackle biological challenges which threaten the sustainability of an industry which supports jobs and economic activity in remote areas.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The benefits will be realised by:

Farmed fish which will benefit from improvements in health and welfare.

Husbandry and veterinary staff who will benefit from access to new tools to maintain and improve the health and welfare of animals in their care.

Aquaculture producers, processing companies and retailers who can expect marketing and price advantages based on reduced losses and more efficient production of fish with higher health and welfare standards.

Supply chain companies who will benefit from opportunities to develop new products and services.

The consumer who will benefit from access to food produced using products which have been developed according to established and assured safety and welfare standards.

How will you maximise the outputs of your work?

We will advertise our capabilities and expertise within our target market so that a wide range of clients can take advantage of these.

We will encourage our sponsors to publish study findings, including negative findings, where appropriate.

We will offer experimental models to research groups for testing novel products. Knowledge of these models will be shared and may be published.

Species and numbers of animals expected to be used

- Other fish: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Typically the product will be administered to experimental fish at the recommended effective dose and by the intended route, for example by voluntary feeding daily for up to 12 weeks or by bath or dip immersion or by intra-peritoneal or intra-muscular injection. In some studies, the product may be administered by oral gavage or intra-venous injection for experimental purposes in order to generate appropriate data for assessment. In target animal safety studies, the product may be administered at higher than the therapeutic dose in order to establish the margin of safety.

Fish will be held in tanks and observed closely during and following dosing and their behaviour and appearance will be assessed against established criteria. Any fish which show abnormal behaviour or appearance will be euthanased and examined.

Fish may be euthanased and sampled during the study. Samples are typically used to measure blood and tissue concentrations of the product at intervals during and following dosing or to assess histological or biochemical changes.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Handling and injection of fish may cause transient pain and distress which will be mitigated by the use of anaesthetics. Fish will typically recover from handling and injection within 24 h.

In safety studies, some fish are expected to show abnormal behaviour and appearance. In a preliminary study, where the safety profile of a product is unknown, there is a greater risk that fish will show more severe clinical signs or mortality in the absence of clinical signs.

Fish will be inspected at frequent intervals so that such signs are identified rapidly and the affected fish will be euthanased within 4 h of developing such signs.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Most fish used in this project will experience no adverse effects other than the pain and distress associated with routine handling for weighing, allocation and dose administration, and will fall within the 'sub-threshold' or 'mild' severity limits. Approximately 15% of fish used in the project are expected to develop more severe or lasting changes in behaviour and appearance requiring euthanasia within the 'moderate' severity limits. Approximately 1% of animals are expected to reach the threshold for 'severe' severity classification or die within a short period in the absence of clinical signs.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 01 October 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The project will test the effects of products on the intended target species which will be a species of farmed fish. Use of experimental animals is required since the behaviour of a new product in the animal and the response of an animal to a particular product cannot be modelled effectively using non-animal alternatives.

In studies conducted for regulatory purposes, the use of relevant animal models is a requirement of the regulatory authorities in order to properly assess the safety and efficacy of the product.

What was your strategy for searching for non-animal alternatives?

Computer simulation models. Cell culture models. Invertebrate models.

Why were they not suitable?

These non-animal models are either not available or not well-enough developed to provide the required high level of confidence in the results specifically as they relate to the target species.

A retrospective assessment of replacement will be due by 01 October 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For each type of study, fish numbers are based on the study design requirements of the regulatory authorities and on published data and/or past experience of appropriate sample sizes.

The estimated total number of animals is based on expected demand and capacity for approximately 32 studies using typical study designs.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

The study designs use prior data to estimate the likely magnitude of variation in response due to random effects and the level of treatment effect which is practically valuable. The discrimination of the study design (ie its ability to distinguish treatment effects from random variation) is maximised by minimising the effects of random variation by the practice of using fish of similar age, source, size range and history, similar experimental tanks and consistent environmental conditions across each study.

Group sizes for voluntary feeding studies are determined by the requirement for fish to show a uniform feeding response. A minimum group size of 20 fish is used in these studies since this is considered to be the minimum necessary to overcome social hierarchy

effects and provide an acceptable feeding response in the majority of individuals in the population.

Smaller numbers of fish may be used where dosing is by immersion, oral gavage, injection or topical administration. In (non-quantitative) safety studies, sample numbers required are those necessary to demonstrate the consistent presence or absence of clinical signs in each exposure group.

In time series pharmacological studies, numbers of fish reflect the number of sample points and the number of individuals required for sampling at each point. Repeat sampling of individual fish was considered but rejected since repeat samples collected from fish which have previously been anaesthetised and sampled may be unrepresentative of the population. In pharmacology/immunology studies which are required to generate quantitative data, numbers of fish used at each point are those necessary to provide an accurate and precise measure of the magnitude of response. This is determined separately for each study using sample size calculation methodology but is typically in the order of 10 fish per time point.

What other measures apart from good experimental design will you use to minimise numbers?

Pilot studies may be used to determine the magnitude of effect of treatment and thereby the number of animals/samples necessary in pivotal regulatory studies.

A retrospective assessment of reduction will be due by 01 October 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

The preliminary safety test aims to establish, using small numbers of animals, that exposure to the product at the proposed dose will not cause pain, suffering, distress or lasting harm in subsequent larger studies. Where relevant, a stepped approach is used to administer the product at the lowest, then medium, and then the highest proposed dose, so that the outcome at one level is known before moving to the next level.

The target animal safety study aims to establish the threshold dose at which the onset of signs indicative of pain, suffering, distress or lasting harm may occur. Animals which reach

this stage will not experience severe or long term effects but will be rapidly identified and humanely euthanased.

Pharmacology/immunology studies aim to generate data on pharmacological behaviour of a product or the biological response to the product at the proposed effective dose which will have been shown to be within the range of safe doses.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The species and life stages used are those for which the products are being developed. Reliable data is important to ensure the safety of farmed fish and the consumer. Less sentient animals have not been shown to provide data which can be reliably extrapolated to the target animals.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Frequent monitoring.

Refinement of criteria used for making, describing and recording clinical signs.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

European Medicines Agency (2011) Guideline on the design of studies to evaluate the safety and efficacy of fish vaccines. EMA/CVMP/IWP/314550/2010. 10 pp.

European Medicines Agency (2011) Guideline on demonstration of target animal safety and efficacy of veterinary medicinal products intended for use in farmed finfish. EMA/CVMP/EWP/459868/2008. 14 pp.

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed) 2018. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018; 16(5): 5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed) 2017. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017; 15(10): 5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

US Food and Drug Administration Center for Veterinary Medicine 1994. Guidance for Industry #53 Evaluation of the Utility of Food Additives in Diet Fed to Aquatic Animals

VICH-GL43 Target Animal Safety for pharmaceuticals

VICH-GL46 Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food producing animals: metabolism study to determine the quantity and identify the nature of residues

VICH-GL57 Studies to evaluate the Metabolism and Residue Kinetics of veterinary drugs in food- producing species: marker residue depletion studies to establish product withdrawal periods in aquatic species

The European Pharmacopoeia (Ph. Eur) 10th edition. Council of Europe, Strasbourg. 2020.

NC3Rs ARRIVE guidelines. https://www.nc3rs.org.uk/arrive-guidelines

Festing, M.F.W., Overend, P., Borja, M.C. and Berdoy, M. (2016). The design of animal experiments. 2nd Edition. Laboratory Animals Handbook No. 14.

Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H. & Turnbull, J. F. (Eds.) (2018). Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare 351pp.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

NC3Rs Newsletter and Website.
REDACTED 3Rs Group.
Relevant training courses.

Communication with sponsors and colleagues working in the field.

Explain the choice of species and the related life stages

The fish species and life stages used in this project are representative of the farmed fish species and life stages for which the products are being developed.

The developers of the products and Licensing Authorities responsible for approval of new products require data from these target species for decision-making and formal regulatory assessment.

A retrospective assessment of refinement will be due by 01 October 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

44. Safety Pharmacology

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	juvenile, adult
Rats	juvenile, adult
Guinea pigs	juvenile, adult
Beagles	adult
Pigs	adult
Cynomolgus monkeys	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The objective of the project is to evaluate the potential of new human medicines to produce unexpected and/or undesirable effects in test animals. The work is required for new medicines, for the safety of human volunteers and patients who will also take the medicines, and it is designed to meet the requirements of regulatory bodies in Europe and elsewhere, who must agree to the sale and use of drugs.

A retrospective assessment of these aims will be due by 14 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?

- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

As well as assuring the safety of human volunteers and patients, the successful conduct of tests will help bring to market those materials which are safe and shown to be effective in the treatment or prevention of human diseases. Without these studies, progression of new medicines to early human studies and to patients could not occur in the current regulatory framework.

What outputs do you think you will see at the end of this project?

Data collected will include information on how various body systems essential to life are affected by potential new medicines, including effects on heart rate and rhythm, blood pressure, breathing rate and rhythm, changes to brain outputs and reflexes, changes to kidney function, changes to intestinal function. The data will be collected to the standards required by government regulators in the UK, Europe and elsewhere, for identifying and excluding inappropriate medicines due to safety concerns, and enabling marketing of successful medicines.

Refined methods of conduct of specific data collection processes may be developed during the course of the project.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Our clients, typically commercial drug companies, will benefit from the provision of high quality of data. This will to help them in their work to new and better medicines, to discontinue development of inappropriate medicines or to understand and manage the risks of new medicines prior to dosing in humans.

Work on this project may also provide data to inform ongoing human clinical trials.

Enabling development of successful medicines will benefit society through diagnosis, treatment or prevention of disease.

Identification of adverse effects can prevent future harms to human volunteers or patients by resulting changes to medicine development programmes.

The wider scientific community may benefit from publication of refined approaches to animal use.

How will you maximise the outputs of your work?

In house collaborations and information exchange with others within the organisation worldwide, identifying successful and unsuccessful approaches.

Collaboration with clients (knowledge gained on products).

On-going collaboration with NC3Rs, resulting in publications (see references).

Hosting scientists and animal carers from other establishments, including universities, to promote best practice.

Presenting outputs at scientific conferences (eg Safety Pharmacology Society conference), user group meetings and Universities REDACTED

Species and numbers of animals expected to be used

- Beagles: 60
- Pigs: 80
- Cynomolgus macaques: 50
- Rats: 5000
- Guinea pigs: 150
- Mice: 1000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Animals will be given potential new human medicines by the same method that people will be given them - such as by mouth, by injection or by inhaling them. Inhalation of drugs generally requires that animals are made accustomed to close restraint in a purpose-made device, and/or are made accustomed to wearing a mask while breathing the medicines. Drugs will normally be given once only at one or more dose levels, and tests will be conducted to look at how the drugs affect the body systems, mainly the heart and lungs, the brain and central nervous system, the kidneys and the stomach and intestines. Some animals will have surgery conducted, under anaesthesia, and with use of pain relief, to allow the use of sensors to provide tests results automatically, such as heart rate or blood pressure. Some animals will be used on more than one occasion; typically this relates to animals which have undergone surgical preparation, as this approach minimises the total number of animals which undergo surgery.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Surgery can cause some discomfort, but this is prevented or minimised by use of appropriate anaesthetics drugs and pain relief. Pain or discomfort from procedures such as injections is likely to be very short-lasting. Behaviour may be affected by the drugs being given, and this may be measured by, for example seeing an increase in heart rate, but the effects are expected to be short-lived, perhaps up to 1-2 hours after giving the drugs.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The harms described above are expected to fall within the mild category for most cases. Where surgery is performed, as described, this would be noted as moderate severity, and may involve about 10% of the total number of animals. If drugs cause some change in behaviour or measured activity such as heart rate this may be considered as having a moderate effect in some animals, estimated at about 15% of the total number based on previous experience.

What will happen to the animals at the end of the study?

- Used in other projects
- Kept alive

A retrospective assessment of these predicted harms will be due by 14 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

While non-animal methods are useful to identify test item effects on individual cell types and on individual chemical pathways, they are currently not able to predict effects on whole body systems or to provide information on how much of a medicine is absorbed. It is not currently possible to acquire all of the information on how the body systems such as the heart , brain and lungs may be affected by new drugs, without using animals. This information is essential, to protect human volunteers and patients. The protocols described in this project are in accordance with regulatory guidelines, and are expected to be performed before government authorities will authorise giving new experimental medicines to people.

What was your strategy for searching for non-animal alternatives?

Although non-animal studies are part of a programme of work when developing new medicines, it is still considered essential by scientists and government regulators, to do work using animals, which this project describes.

Why were they not suitable?

There currently remains general scientific agreement that to protect human volunteers and patients, non-animal alternatives do not, as yet, provide enough information to replace animal studies.

A retrospective assessment of replacement will be due by 14 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimates are based on analysis of use of animals in an existing licence authorising work for the same purpose, combined with anticipated upcoming studies.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Statisticians have performed calculations for each of the study types in this project and have provided advice on experimental design for all standard study designs within the project. This ensures that studies are correctly designed to meet the study objectives. Statisticians will be consulted on a case by case basis for any study that requires a non-standard design.

What other measures apart from good experimental design will you use to minimise numbers?

Pilot studies will be used to investigate the potential of new designs to improve outcomes. These could include evaluation of new implantable devices, ways to analyse the data, or additional tests to conduct, leading to improved data quality.

A retrospective assessment of reduction will be due by 14 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Dosing of test items are usually by the same routes as the medicines would be given to humans; all very well established and common methods for the species to be used. Volumes of drugs to be given are in line with published guidance on minimising discomfort, and/or are known to cause minimal discomfort based on extensive experience at the site.

Blood sampling follows published guidance on suitable volumes which can be taken while minimising harms to animals.

Surgery is conducted with expert veterinary involvement in the creation of suitable regimes for anaesthesia and post-surgical pain relief.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The species used are selected based on known standards of outcome which will answer the scientific questions; known industry and regulatory standards of models which will answer both scientific and regulatory questions; to enable comparison with other data being generated in the same species as part of the safety assessment of potential new medicines. Response to tests is assessed over a time period which would make continued anaesthesia impractical, and would interfere with the outcome in some circumstances.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Monitoring of on-going procedures is refined for cause as any concerns are identified; for example additional assessments may be included based on initial outcomes. The surgery and anaesthesia/pain relief protocols used in the programme undergo continual assessment and refinement to improve outcomes.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Dose volume and blood volume limits agreed with the animal welfare and ethical review body are based on the 2001 publication of Diehl et al: A good practice guide to the administration of substances and removal of blood, including routes and volumes.

Welfare end-points are developed in general line with publications on the topic, including the NC3Rs document from 2010 on dose level selection for regulatory toxicology studies.

Non-human primate housing is in compliance with the NC3Rs document on this topic from 2017.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We are involved with other groups working in the same field, and have participated in analysis and publication of data in the scientific field to assess methods of conduct and

outcomes. The licensee and others at the establishment have been involved with various working groups of the UK National Centre for the 3Rs (NC3Rs), over many years.

Explain the choice of species and the related life stages

Many scientific studies have been conducted to demonstrate that the types of animals to be used in the project will provide results which reflect the likely effects in humans. The way in which each new medicine works in the body will be known, and the animal type(s) to be used will be chosen based on an understanding that the medicine will work in a similar way.

Another big advantage of using the listed animal types is the ability to compare the results with the results of tests in these same animal types which are used in other safety studies conducted to assess other questions of safety, and which are acceptable to the government agencies responsible for authorising use of the drugs in human volunteers and patients. Development of drugs can not currently be achieved without this approval by government agencies in the UK, elsewhere in Europe and in other parts of the world.

A retrospective assessment of refinement will be due by 14 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

45. Sex differences in brain and behaviour

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants.

Key words

Behaviour, Brain, Psychiatric illness, Sex chromosome, Sex differences

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Men and women differ substantially in their behaviour, and their vulnerability to a number of common and disabling psychiatric illnesses. Whilst we know the biology of the genders is distinct, there is little known about the routes through which gender-specific behaviours and disorder vulnerability arise. This project aims to increase our knowledge regarding the biological (particularly genetic and hormonal) mechanisms that may predispose to these sex effects.

A retrospective assessment of these aims will be due by 23 October 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

We are particularly interested in understanding the biological mechanisms by which males are more likely to be diagnosed with developmental disorders (such as autism and Attention Deficit Hyperactivity Disorder) and by which some women develop psychiatric illnesses during pregnancy and after giving birth. Identifying and characterising these mechanisms could potentially reveal new therapeutic targets for use in treating sexually dimorphic brain disorders.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We will use wildtype and genetically altered rats and mice in this work; these species, in which genes can be modified, show considerable genetic and neural homology with humans, key attributes in terms of modelling psychiatric processes dependent upon altered gene function. Moreover, elegant behavioural tasks with established translational utility are already available for these species. We anticipate using ~2,500 mice and 600 rats over the course of the licence.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

The majority of procedures carried out under this licence will be mild, and will cause no more than transient stress or pain; similar to my previous licences, I expect that approximately 80% of procedures to be carried out will be of severity rating ‘mild’, and ~20% ‘moderate’. Novel genetically altered animals may exhibit unanticipated health issues. Administering drugs to pregnant mothers may have mild, adverse effects on the mother’s health, or on rates of pup death/malformation. Transferring litters from genetically altered to wildtype mothers to test genetic mutation effects on mothering may lead to increased rates of pup-killing. In order to motivate performance in some behavioural tasks, a degree of food restriction or water restriction (up to 22hrs per day) will be necessary leading to potential weight loss. A minority of behavioural tasks will be aversively motivated (e.g. by mild footshocks, or by immersion in water) resulting in transient pain or distress. Neuroactive substances may be locally or systemically administered to some animals; these drugs may have mild, transient effects on behaviour. Surgical procedures may result in mild discomfort.

A retrospective assessment of these predicted harms will be due by 23 October 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

We need to use animals in our research for several reasons. First, it is not yet possible to model the complexities of behaviour in isolated tissue systems or by using computer simulations. Second, our rodent lines will, in many cases, serve as direct models for human disease situations. Where our in vivo work suggests underlying molecular/cellular mechanisms for behvaioural abnormalities, these will be further explored in in vitro systems; the results of these studies will help to inform our in vivo work. Here possible, we will ask questions using either healthy human and clinical populations to which we have access, or cellular model systems.

A retrospective assessment of replacement will be due by 23 October 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

We will minimise animal usage through performing power calculations once initial pilot data are available to ensure that we breed and use the minimum number of animals for obtaining a reliable experimental results; we will also use as many animals from each litter as possible (either as experimental subjects or controls). We will continue to improve our behavioural methods such that fewer animals are lost through attrition e.g. failure to learn the task.

A retrospective assessment of reduction will be due by 23 October 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Existing genetically altered rodents to be used exhibit no adverse effects on health/wellbeing; any new mutants created will be monitored closely in consultation with a vet and experienced animal technicians, and. if necessary, will be treated/culled. The majority of behavioural procedures to be used will either be non-regulated, or appetitively-motivated with palatable foodstuffs; the water and food restriction protocols to be used do not result in adverse effects on general health or wellbeing. Where aversive procedures

are used, these will generally be mild and will not result in long-term adverse effects; particular attention will be paid to animals undergoing such procedures. Any drugs given will be non-toxic, and will be given in suitable and minimal volumes of vehicle. All surgery will be performed under aseptic conditions under general anaesthesia, and all efforts will be made to minimise potential associated pain using an appropriate analgesic regime. Where animals exhibit adverse effects on health and wellbeing resulting from the procedures, they will be closely monitored and treated appropriately by Personal Licencees, technicians and/or the REDACTED vet; if these interventions do not improve the animal's condition sufficiently, they will be culled (generally by a Schedule 1 method).

A retrospective assessment of refinement will be due by 23 October 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

46. Targeting RNA metabolism to expand normal blood stem cells and eradicate cancer stem cells in acute myeloid leukaemia

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	embryo, adult, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

We aim to identify regulators of normal blood functions and provide mechanisms through which stem cells undergo leukaemic transformation and become leukaemic stem cells (LSCs). This research will allow us to therapeutically target LSCs/cancer stem cells while enhancing normal blood cell functions.

A retrospective assessment of these aims will be due by 04 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Lifelong blood production critically depends on blood stem cells, also called haematopoietic stem cells (HSCs), which possess the unique ability to generate all types of blood cells. Due to these properties, stem cell transplantation, in which blood stem cells are sourced from adult donors or cord blood, offers optimal treatment for many diseases, including severe autoimmunity disorders, immunodeficiencies, bone marrow failure syndromes and blood cancers. Given the shortage of suitable donors and low numbers of stem cells from cord blood tissue, it is essential to efficiently expand blood stem cells in a dish for widespread clinical applications.

Acute myeloid leukaemia (AML) is a blood cancer in which stem cells acquire mutations and form treatment-resistant leukaemic stem cells (LSCs), which initiate and propagate the disease. Since current therapies often fail to eliminate LSCs, the surviving LSCs cause severe disease relapses. It is therefore essential to identify efficient means of LSC elimination.

What outputs do you think you will see at the end of this project?

This project aims to investigate the effect of inhibiting specific proteins involved in a process known as RNA metabolism on normal and leukaemic stem cells. RNA acts as a messenger, relaying instructions from DNA to help coordinate the production of proteins. This process is crucial as proteins are responsible for nearly every task in the cell. RNA metabolism, where proteins regulate the synthesis and degradation of RNA, is therefore fundamental for the function of cells. We hope to establish the therapeutic potential of inhibiting specific proteins involved in RNA metabolism in order to expand blood stem cells and target LSCs in different subtypes of AML.

Given the shortage of blood stem cells donors, we aim to identify drugs which will efficiently expand blood stem cells in a dish for multiple clinical applications, such as stem cell transplantation. Importantly, these drugs will also be used to eliminate LSCs in different blood cancers, providing an essential new therapy for AML patients. Bearing in mind that LSCs share similarities with other cancer stem cells, we will work with our collaborators to explore the broader applications of our findings in other cancers. At the end of this project we expect to publish multiple high-impact papers in the stem cell, cancer and leukaemia biology fields.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Understanding leukaemic/cancer stem cell biology is incredibly important as it has broad ramifications for several fields including oncology, haematology, and drug discovery. Eradication of leukaemic/cancer stem cells is essential to develop effective new treatments in blood and other cancers. Furthermore, due to the shortage of blood stem cells from donors, the expansion of blood stem cells for multiple clinical applications including transplantation remains an important research goal. Our research is therefore of an immense strategic importance and addresses key areas of unmet clinical needs.

The key beneficiaries are:

AML patients: We aim to achieve efficient eradication of AML leukaemic stem cells thus providing curative treatments for patients. Patient benefit will depend on the efficient translation of our work to the clinic. We have strong links with the pharmaceutical industry, as well as clinical haematologists around the UK and will work closely with them to achieve this as rapidly as possible.

Patients with other blood malignancies: Once we provide a proof of concept in AML treatments, we will test whether similar therapies can be applied to other blood malignancies (e.g. chronic myeloid leukaemia, myeloma, lymphomas).

Cancer patients: In the longer term, we will collaborate with our colleagues focusing on different cancers to test whether our treatment strategies can be employed in other cancers, creating new treatment options.

Patients requiring stem cell transplantation: Achieving blood stem cell expansion in a dish will be a breakthrough for patients who require a stem cell transplantation. This includes patients with many disorders including severe autoimmunity disorders, immunodeficiencies or bone marrow failure syndromes.

How will you maximise the outputs of your work?

We will strictly adhere and support our funders' policy for research data sharing and management. Our data will be presented during multiple conferences/workshops and published in open-access high quality journals. After publication, data will be deposited in an open access digital repository. Published materials/reagents/mice will be available upon request to allow for collaboration and dissemination of new knowledge. Together, our data will be safely stored to ensure their longevity and that they can easily be shared, uploaded or reanalysed by everyone.

Species and numbers of animals expected to be used

- Mice: 20,000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Most of our mice will be used for breeding to generate cells, tissues and organs lacking genes of interest. The mice will be humanely culled to obtain this research material, typically at the age of 8-12 weeks. We will also breed our recipient mice which will undergo bone marrow or stem cell transplantation. In this procedure, recipient mice undergo an irradiation procedure to remove their bone marrow cells, and are then injected with new stem cells or bone marrow cells. These recipient mice will be kept for 16 weeks after transplantation. In some occasions, we will cull these mice, collect the bone marrow and

re-transplant the cells into other recipient mice, which will be also kept for 16 weeks. This experiment, known as a secondary transplant, is required to test the self-renewal capacity of blood stem cells, i.e. their ability to continually generate all types of blood cells. Some of our transplanted mice may develop blood cancers. All mice will be very carefully monitored and those showing unexpected side effects will be culled. However, a very small percentage of sudden deaths may happen (despite an intensive program of care and welfare). All mice will be humanely killed at the end of each experiment.

In some cases, we will need to withdraw small quantities of blood from both non-transplanted and transplanted mice by inserting a fine needle into a vein. This causes short-lasting discomfort, similarly to patients who give a blood samples for testing.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

The expected impacts and adverse effects for animals in this project vary depending on their use. In the majority of cases, breeding involves no adverse effects, pain or suffering. In some rare cases we may have to breed healthy mice that are susceptible to leukaemia. These mice will be carefully monitored on a daily basis, so their suffering will be minimised.

In transplantation experiments, after irradiation, animals may experience weakness, modest weight loss and some abnormal behaviour, such as withdrawal from the group. Animals may also experience some pain, and may be given pain killers by veterinary staff. These adverse effects, caused by the irradiation itself, normally last for several days and then the animals fully recover. During this period (5-14 days after irradiation), mice will be scored every day according to the scoring system we have developed and refined. This system gives us an objective way of assessing animal health, and also allows us to clearly identify and apply a humane killing point at the earliest opportunity, both to achieve our scientific goals and to minimise suffering.

Some of our transplanted mice may develop blood cancers. Mice will be very carefully monitored and evaluated, and any animals showing the earliest clinical side effects will be culled. However, due to the nature of the disease, a very small percentage of sudden deaths may happen. All mice will be humanely killed at the end of each experiment.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

A large number of our mice will be used for breeding, where we expect to see no adverse effects. When breeding mice susceptible to leukaemia (which covers approximately 4,000 of our mice for the period of 5 years), approximately 10-20% of mice will experience adverse effects such as weakness, modest weight loss and some abnormal behaviour, such as withdrawal from the group.

We plan to use approximately 5000 mice to investigate the functions of normal and leukaemic stem cells following gene deletion. Some mice in these experiments will develop leukaemia, but due to close monitoring, the vast majority of mice will be culled

before they develop significant adverse effects. We estimate that 10-25% of mice may experience adverse effects as a result of leukaemic disease.

Approximately 10000 mice will be used for transplantation assays to study functions of normal and leukaemic stem cells. Of these experiments, approximately 50% will be injected with leukaemic cells, and we expect 20-80% of those to suffer adverse effects. Deaths related to leukaemia will be less than 5% of all mice in these experiments.

What will happen to the animals at the end of the study?

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 04 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

In some instances, the use of animals is currently unavoidable as many facets of stem cell and leukaemia biology can only able to be studied in animals, such as mice, where these cells and diseases naturally occur. For example, the current knowledge does not allow us to properly culture blood stem cells in a dish – once plated into a dish they die and lose their potential. Current culture conditions also do not allow us to test many key properties of leukaemic cells, including their ability to cause leukaemic disease. Therefore, at the moment, many important experiments need to be conducted in mice. Additionally, given that our ultimate plan is to take our research into clinical trials, where new treatments are tested in human patients, appropriate validation of the therapeutic targets we investigate has to first be done in mouse models.

What was your strategy for searching for non-animal alternatives?

Where possible, we strive to use non-animal alternatives in our research. We often use stem cells from human cord blood tissue instead of mice. We also use human bone marrow-derived cell lines in dish- based tissue culture experiments to obtain information about human leukaemic disease and to test the role of genes of interest in AML, and we will continue to do so.

Notably, we are very active in developing techniques that would improve our ability to study aspects of leukaemia in a dish. In a recent publication, we have developed machine learning algorithms and a high-throughput tissue culture-based screen for drugs against leukaemia, involving the testing of various drugs against leukaemic cells in a dish.

Transplantations of blood stem cells or leukaemic stem cells into recipient mice are essential experiments for the study of stem cell and leukaemia biology. However, these experiments require large numbers of mice, both mouse donors, from which blood cells are derived, and mouse recipients, into which blood cells are injected. Whenever possible, we replace mouse transplantation with dish-based tissue culture experiments, so called long-term culture-initiating cell or colony-forming cell assays which require very few animals.

Moreover, to study genetic pathways in stem cells, whenever possible, we perform predictions using computational software instead of using animals. We have a dedicated bioinformatician (specialist computer scientist) in our group whose role is to study sequencing data from leukaemia patients to obtain maximal information about the biology of the disease. While these are strong predictions, our findings need to be validated in animals before we can move our research into clinical trials with human patients.

Why were they not suitable?

Blood stem cells and leukaemia stem cells reside in bone marrow niches, very specialised pockets within the bone marrow which provide stem cells with a specific microenvironment composed of multiple biological factors that support their functions. Culture conditions in the dish fail to reproduce this complex microenvironment. Indeed, when exposed to culture, stem cells lose their activity and adopt characteristics of non-stem cell types, making them unusable for research. As such, non-animal alternatives are sometimes unsuitable and we must use animals to accurately study stem cell and leukaemia biology in a whole animal context.

A retrospective assessment of replacement will be due by 04 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of mice are carefully planned based on our long-term experience and consultations with our expert collaborators. An experienced statistician in our group performed careful estimations to ensure that the number of animals used in our experiments is the minimum number required to generate statistically significant results (i.e. statistically convincing results). Designing animal experiments to produce statistically significant results means that we can generate more powerful data. This data can then be translated into clinical applications, such as curative AML therapies, as quickly as possible.

We plan to breed and generate 20,000 mice for this project for the period of 5 years. 5,000 of these mice will be used for cell-based tissue culture assays. 5,000 will be used to study stem cells functions, and 10,000 will be employed to examine stem cell functions upon transplantation. In all experiments we will use mice of both sexes to avoid having to cull a surplus of mice of a particular sex.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

As key means of achieving reduction we have and will continue to carefully design our experiments and mindfully estimate the number of animals required in order for the results of our experiments to be statistically convincing. To minimise the number of animals used in each experiment, we will use power calculations, calculations that allow us to gauge how statistically convincing a given result is. For example, in preliminary studies where we are trying to determine the most effective number of cells, or drug dosage to use, no more than 3 animals per group will be used when possible. Experiments will be carefully planned to maximise the information obtained per animal and thus limit the subsequent use of additional animals. Experiments requiring cells from animals will be carefully optimised in order to minimise the number of animal cells required.

What other measures apart from good experimental design will you use to minimise numbers?

An important means of achieving reduction is to apply the most efficient breeding strategies. My group has extensive expertise with mouse colony management (i.e. monitoring large cohorts of mice) and we will strive to employ the best breeding schemes. We will replace breeders before their reproductive performance declines, and non-productive breeders will also be replaced.

My group is fully aware of the ARRIVE guidelines and experimental design tools provided from NC3Rs. As such, we employ the optimal experimental designs to implement the 3Rs and optimise the number of mice for each experiment.

Importantly, experiments will be carefully planned to maximise the information obtained per animal and thus limit the subsequent use of additional animals. For example, haematopoietic organs, i.e. organs involved in the blood system, including bone marrow, lymph node and spleen cells will be stored and used for multiple experimental purposes. All experiments requiring cells from animals will be carefully optimised in order to minimise the number of animal cells required.

A retrospective assessment of reduction will be due by 04 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the

mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

In the majority of our experiments we will study mice lacking genes of interest specifically within the blood system. These 8-12-week old mice will be analysed to study the haematopoietic organs (bone marrow, spleens, thymi and lymph nodes), which can also be used for tissue culture or transplantation experiments. In some cases, we will age these mice for 60 weeks to study the role of genes of interest in the ageing of the blood system. These experiments do not involve any invasive procedures and therefore any pain, suffering or distress will be minimal.

Given that the major goal of our work is to study leukaemia, in some cases we will need to inject leukaemic cells into recipient mice. Following this, these mice will develop leukaemia. In 50% of cases they will be analysed before they develop clinical symptoms of leukaemia. In another 50% of cases, when the progression of leukaemia is of scientific interest, mice will be monitored and culled at a point such that our scientific research is achieved, but pain, suffering and distress is minimised.

All these models are the current state-of-the-art and the gold standard in cancer and stem cell research. If/when other improved models become available, we will immediately implement them in our research. We are very active in trying to refine our procedures. For instance, at the moment bone marrow imaging techniques are not developed sufficiently. However, we are working with key experts in the field to try to optimise these approaches to be able to non-invasively image cells in the bone marrow and monitor stem cells without having to sample blood or bone marrow from mice.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Given that our aim is to target human cancer stem cells in leukaemia or expand adult stem cells, we will need to employ mice for these purposes. Mice and humans share the same genes of interest and the cellular processes involved in stem cell biology and leukaemia are very similar. As such, mice are ideally suited for our work as they allow us to gain insight into human stem cells and leukaemia biology without having to work on human patients. Furthermore, all the reagents necessary for our research are mostly developed for the mouse and human systems. Other less sentient animal models (e.g. flies, fish or worms) are excellent model systems to study some conserved biochemical pathways, but do not replicate complex human or mouse blood stem cell and leukaemia biology. In fact, there are no models of leukaemia in these species. However, we do collaborate with several experts around the world to obtain maximum relevant information from less sentient animals.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

To ensure technical competence, all staff will be directly supervised by the project license holder. To minimise infections of mice with impaired immune systems, the animals will be housed in barrier caging under sterile conditions and handled in a sterile environment.

Whenever appropriate, in order to prevent pain, pain killers will be given as directed by veterinary staff. Genetically modified animals exhibiting any unexpected pain or suffering will be humanely culled. For protocols involving bone marrow transplantation and leukaemia experiments, we have developed and successfully used a stringent scoring system which allows immediate identification of mice displaying adverse effects. This system gives us an objective way of assessing animal health, and also allows us to clearly identify and apply a humane killing point at the earliest opportunity, both to achieve our scientific goals and to minimise suffering.

Over the last 10 years we have optimised and refined many procedures. For instance, as mentioned above, we have optimised a clinical scoring sheet allowing us to rapidly identify any animals which are likely to develop clinical symptoms of leukaemia or post-irradiation sickness, and cull them humanely before they start suffering. Importantly, to further refine transplantation procedures using human AML cells or cord blood stem cells, we are in the process of optimising transplantation procedures using mice lacking aspects of their immune system, so-called immunodeficient mice.

Notably, our current transplantation experimental protocol requires that the recipient animals undergo an irradiation procedure. Without irradiation, the immune system of the recipient mice would reject all donor cells, rendering the experiment ineffective. Irradiation procedures allow us to preform successful transplantation experiments, generating important data. However, irradiation can have unwanted health effects on animals, which is why we are seeking to replace irradiated recipients with immunodeficient mice in our transplantation experiments. Due to their impaired immune system, immunodeficient mice will already not reject donor cells, and so do not require irradiation. Optimising transplantation experiments using immunodeficient mice will reduce pain and suffering, and so will be a major refinement to our procedures.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Our experimental designs are based on our long-term experience in this field (we have already refined many protocols and experimental designs) and peer-reviewed high-quality literature. We always seek to apply the most refined methods which are published in the field. We also base our knowledge on ample literature disseminated by NC3R and interactions with many expert colleagues and collaborators. We will always continue to refine our protocols based on the literature and knowledge exchanged with skilled collaborators and we are very proactive in this area.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Many advances in cancer research, including advances in mouse cancer models, are disseminated during scientific conferences and seminars, which we frequently attend. Further, members of my group frequently attend 3Rs events organised by NC3R, and we continue to update our knowledge through the literature NC3R disseminates. We also have an extensive network of local and international collaborators who use similar state-of-the-art models, and by frequent exchange of information we will always stay informed about the best advances in the field. Given that our group is extremely committed to the implementation of the 3Rs, we will utilise any useful knowledge learned during these events and the literature to improve mouse procedures, apply petri dish-based models where possible, improve statistical methods, and minimise pain and suffering of our experimental mice.

Explain the choice of species and the related life stages

Many dish-based or computational-based experiments are unsuitable to study human blood stem cells or cancer stem cells in leukaemia. As such, we need to employ mice to recapitulate these complex biological processes. Our lab uses mice for research because mice and humans share the same genes of interest as well as the cellular processes involved in stem cell biology and leukaemia.

In general, we will use 2 types of mice.

Firstly, in the majority of our experiments we will study genetically altered mice - mice lacking genes of interest specifically within the blood system. In our preliminary experiments, we found that removal of these genes results in the expansion of normal stem cells and elimination of cancer stem cells. To further explore these results, and prepare our data for publication and translation into clinical trials, we plan to examine the impact of removing these genes from mice. Typically, 8-12 week-old mice will be culled to obtain haematopoietic organs, which will be used for analyses, dish-based tissue culture or transplantation experiments. In some cases, we will age these mice for 60 weeks to study the role of genes of interest in the ageing of the blood system.

Secondly, we will use non genetically altered mice as recipients of bone marrow or stem cell transplantation. Transplantation of blood cells into recipient mice is the gold standard way to analyse the activity of normal stem cells or leukaemic/cancer stem cells. These experiments allow us to study how normal stem cells regenerate blood or how leukaemic/cancer stem cells generate leukaemia.

A retrospective assessment of refinement will be due by 04 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

47. Testing of Veterinary Immunologicals

Project duration

5 years 0 months

Project purpose

- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	juvenile, adult
Guinea pigs	juvenile, adult
Rabbits	juvenile, adult
Domestic fowl	neonate, juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

Quality Control testing of in-process and final product (vaccine) test samples to ensure they meet set specifications and requirements before they are released for sale. These tests ensure the products are safe, have the right quality and are efficacious.

A retrospective assessment of these aims will be due by 06 July 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Vaccines protect animals against serious and potentially fatal diseases. The vaccine formulations have been used for several decades and reliably provide excellent protection from disease. Use of these products worldwide is established veterinary practice and enhances the welfare of animals by controlling animal disease or human food poisoning. Laboratory testing is a legal requirement to ensure consistency of the vaccine and ensures that any substandard product is not released for use.

Over 1.93 Billion chickens, sheep, pigs and cattle will be protected from disease by the vaccines tested under this project.

What outputs do you think you will see at the end of this project?

The manufacture and marketing of several different farm-animal vaccines that are consistent, safe and reliable. Use of these vaccines will enhance animal welfare due to reduced disease incidence and contribute to food safety by reducing transmission of Salmonella in eggs.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Vaccines protect animals against serious and potentially fatal diseases. The vaccine formulations have been used for several decades and reliably provide excellent protection from disease. Use of these products worldwide is established veterinary practice and enhances the welfare of animals by controlling animal disease or human food poisoning. Laboratory testing is required to ensure consistency of the vaccine and ensures that any substandard product is not released for use.

It is estimated that over 1.9 billion farmed animals will be protected from disease by the vaccines tested and manufactured under this project, equivalent to protecting greater than 20,000 animals for each animal used in the five-year project

How will you maximise the outputs of your work?

Due to the unique business and confidential nature of this work it is difficult to share experiences apart from collaboration with other company production sites.

Species and numbers of animals expected to be used

- Mice: 74500
- Guinea pigs: 9100
- Rabbits: 8600
- Domestic fowl: 2000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Injections of vaccinal components and final vaccine products with a result being obtained by measuring antibody responses and/or absence of any adverse side-effects.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Most animals may experience short-term discomfort following injection. Analgesia will be given if discomfort is expected to be of longer duration

Possible signs of clinical disease. These animals are euthanased at the onset of clinical signs to prevent any suffering.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

- Domestic fowl: Mild procedure.
- Rabbits: Mild procedure.
- Guinea Pig: 1.2 % Mild procedure.

All other Guinea Pigs will show moderate to severe clinical signs. These animals will be supported with analgesia and close monitoring. A significant majority are euthanised as soon as the clinical signs are displayed.

Mice: 50% are expected to show moderate to severe clinical signs, however, with close monitoring a significant majority are euthanised as soon as these signs are displayed.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 06 July 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animal tests will only be used where it is a legal requirement to ensure the final released products are efficacious and safe. Where legislation permits and there are alternative non-animal tests available they will always be used.

What was your strategy for searching for non-animal alternatives?

Laboratory tests, e.g. ELISAs are used for quantification of many of the in-process and final components of the vaccines produced. Over recent times animal tests have been replaced by laboratory methods (for example cell line assays and Antigen mass assays) and further research is ongoing in these areas.

Why were they not suitable?

Alternatives are suitable and are used for several of the tests but some vaccinal components have proven difficult to identify and their assay using in-vitro models is not currently feasible. We are actively pursuing replacement of the remaining animal tests for the future.

A retrospective assessment of replacement will be due by 06 July 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers are based on what will be required for legal and regulatory testing of the products that are planned to be manufactured and tested over the next five years. These numbers include all stability work that is scheduled.

The estimated number of mice to be used under Protocol 1 is 55000. The testing performed under this protocol includes all work performed on in-process antigen toxoids and toxins (4 different tests are performed on 15 different species of antigen - each test uses upto 10 mice per sample as well as control mice which are typically shared between samples) to determine the overall yield of the sample and ensure that the antigens have fully detoxified before they are used in the blending of final vaccines.

These toxoid antigens have a long shelf life which ensures that all samples produced and tested are utilised and none are discarded.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

The numbers of animals involved are primarily dictated by testing regulatory standards but there may be areas where less animals could be used in the future by a process of statistical and scientific analysis of data and this is an avenue that is currently being actively pursued.

What other measures apart from good experimental design will you use to minimise numbers?

The number of animals is prescribed by regulatory requirements and future reduction using in-vivo replacement or statistical and scientific arguments will be investigated.

A retrospective assessment of reduction will be due by 06 July 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Animals are used for some of the testing to ensure that batches of veterinary medicines are efficacious, safe and consistent, as dictated by legal regulatory requirements.

Some tests may cause animals to develop significant clinical symptoms. By regular clinical monitoring performed at critical periods by experienced, trained and competent personnel, any suffering will be minimised by prompt euthanasia.

Where relevant, early recognition of a Humane Endpoint (HEP) is essential to the conduct of this work and any animal approaching a recognised HEP for the specific test will be promptly euthanased.

If a test is predicted to cause some discomfort, suitable analgesia will be given prior to procedures being performed.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

Legal regulatory requirements dictate the species used.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Some tests may cause cause animals to develop significant clinical symptoms. By regular clinical monitoring performed at critical periods by experienced, trained and competent personnel, any suffering will be minimised by prompt euthanasia.

Where relevant, early recognition of a Humane Endpoint (HEP) is essential to the conduct of this work and any animal approaching a recognised HEP for the specific test will be promptly euthanased.

If a test is predicted to cause some discomfort, suitable analgesia will be given prior to procedures being performed.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

The support network, including Named Persons actively disseminate information posted from bodies such as the IAT, Home Office, NC3R's, RSPCA, LASA etc.

The NVS and his support team are members of the VOLE e-mail network and the NVS is a council member of the Laboratory Animals Veterinary Association (LAVA). The veterinary care team have the ability to consult with an extensive network with close connections with the relevant and experienced colleagues.

The NACWO and the animal care team actively pursue exploring and applying new initiatives for refinement and the well-being of the animals they care for.

All staff are keen to attend events where there is opportunity for education and networking to enhance the high standard of care for animals.

Frequent updates are effectively disseminated within our organisation by the HOLC, and by all present at regular AWERB meetings.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Active involvement and discussion with Animal Technology resources on an ongoing basis.

A continuing active dialogue regarding animal welfare and experimental refinement between the animal care staff, Project Licence holder and Named Persons.

The AWERB is regularly informed and kept up to date with any developments and any concerns are actively discussed.

Explain the choice of species and the related life stages

Small laboratory species are used to evaluate the safety and efficacy of vaccines for farm species.

A retrospective assessment of refinement will be due by 06 July 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

48. The molecular regulation of the immune response

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Mice	adult, juvenile, pregnant, embryo, neonate

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

We aim to understand how the immune and inflammatory response is regulated with a view to generating novel insights into immune and inflammatory diseases hopefully leading to new therapies.

A retrospective assessment of these aims will be due by 22 November 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The molecules that we study, which are called chemokines, are central to the ability of white blood cells to move to sites of inflammation. Normally this is a healthy process but when this is dysregulated, these white blood cells can cause extensive damage leading to major diseases such as cancer, rheumatoid arthritis and asthma. Our aim is to try to understand how these molecules function with a view to helping to identify novel methods for therapeutically targeting them in inflammatory diseases.

What outputs do you think you will see at the end of this project?

We are interested in trying to understand how inflammation is regulated. Unfortunately this is currently poorly understood and we believe that the proposed programme of work, using existing and novel mouse modelling techniques, will provide unprecedented insights into the inflammatory response. Specifically we will define how molecules called chemokines are involved in orchestrating the inflammatory response. The data to be generated will shed light on the roles for chemokines and their receptors in basic inflammation as well as in inflammation-dependent cancer models and models of viral and bacterial infection. Our data will also have implications for our understanding of chemokine and chemokine receptor involvement in a wide range of human pathologies with the ultimate hope that this will lead to the development of novel therapeutics. We therefore propose that our studies will be of societal benefit in terms of enhancing understanding of inflammatory disease and improving options for therapy.

In addition we are interested in molecules called atypical chemokine receptors which sit in the placenta and are crucial for stopping chemokines moving from the mother to the embryo. When this mechanism fails, chemokines enter the embryonic circulation and can disrupt aspects of embryonic development. We therefore propose to study this mechanism in detail with a view to understanding the implications of disruption of atypical chemokine receptor function on embryonic health and development. We propose that these analyses will be important in helping to understand the molecular basis for a number of known illnesses and developmental abnormalities that result from the effects of maternal infection and inflammation on the embryo.

At all points we will publish our data in peer-reviewed journals and make it freely available to the scientific community through open access publications.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The beneficiaries from our research fall into 2 broad categories:

as chemokines are essential for the establishment of inflammation in many inflammatory diseases, as well as in cancers, an enhanced understanding of their role in these pathologies will highlight novel opportunities for therapeutic intervention. We believe, therefore, that a major benefit of our research will be the development of novel therapies for currently untreatable inflammatory diseases and cancers.

the characterisation of the importance of atypical chemokine receptors for blocking chemokine movement from the mother to the embryo will help identify potentially novel mechanisms underpinning developmental abnormalities in children. Specifically we will be interested in looking at whether our molecules are the basis for what is called maternal

immune activation in which offspring, born to mothers experiencing inflammation, display neuropsychiatric disorders and altered immune health.

How will you maximise the outputs of your work?

We are very well integrated in the international chemokine and immunology communities and take every effort to present our research to colleagues throughout the world. In addition we publish our research in high profile international journals making them available, through open access publishing, to any interested parties. Furthermore we put a lot of effort into public engagement and present talks to schools and lay groups about our research.

Species and numbers of animals expected to be used

- Mice: 25,000 over 5 years

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Typically, animals will be treated with an inducer of inflammation and their response to that inflammatory agent assessed. Inflammatory responses to viral and bacterial pathogens will also be measured as will the involvement of chemokines and their receptors in tumour-associated inflammatory responses. In addition they may be injected with molecules capable of regulating immune response to see what effect this has. In a small number of cases we will surgically implant capsules capable of releasing immune and inflammatory mediators over time. This has the advantage of avoiding repeated injections into the mouse.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Depending on the protocol, animals may experience mild pain, weight loss (not to exceed 20%) and tumour development (tumours not to exceed 1.2 cm in maximum diameter). We also suspect that some of the offspring of mothers treated with inflammatory and immune regulators may display abnormal behaviour although we do not predict that this will be associated with pain. Animals will never be allowed to experience pain or display abnormal behaviour longer than is necessary for the analysis. Weight loss and tumour development have strictly regulated endpoints beyond which animals will not be allowed to remain on the protocol.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The majority of protocols are associated with moderate severity and even for these, moderate severity will only be experienced by a minority of mice. We have 3 protocols which are associated with a severe rating and these relate to infection of mice with Influenza and/or bacteria, or virulent encephalitic viruses. Again not all mice will experience severe adverse effects and mice will be monitored throughout and removed from the study immediately upon appearance of such severe effects.

What will happen to the animals at the end of the study?

- Killed

A retrospective assessment of these predicted harms will be due by 22 November 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The immune and inflammatory response is complex and involves the interplay of numerous cell types. It is not currently possible to adequately recapitulate this response using cells in vitro. Only in the intact animal will the correct combination of cells and circumstances be present to faithfully reflect the biology of the immune and inflammatory response. In addition, there are a wide range of reagents and molecular approaches available to manipulate the immune and inflammatory response in animals to allow us to detail mechanisms. Again such analyses are currently not possible using non-sentient in vitro alternatives.

What was your strategy for searching for non-animal alternatives?

Wherever possible we use in vitro approaches. In terms of Objective 2, we will use in vitro trophoblast cell lines to analyse some mechanisms. In addition when looking at simple responses of immune and inflammatory cells we do, wherever possible, use cell lines or in vitro derived immune cells.

Why were they not suitable?

These cell lines are not suitable for the majority of analyses because they exist in isolation in vitro in contrast to the in vivo situation in which they are required to interact with a wide array of different cell types to mediate the overall immune and inflammatory response.

A retrospective assessment of replacement will be due by 22 November 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

This number is based on previous HO animal returns and are breeding records. In general we use between 4000 and 5000 mice per year.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?
We routinely perform power calculations for experiments in which we have limited experience. For most experiments we have many years experience of optimising group numbers. Where power calculations are required we also seek expert advice from colleagues at REDACTED

What other measures apart from good experimental design will you use to minimise numbers?

In all experiments, mouse numbers will be determined to produce statistically robust data and we will not use any mice beyond the numbers required to this end. In addition, for all experiments, as well as harvesting key tissue for analysis, we will store other tissues to allow us to re-analyse them if relevant to addressing secondary questions. This will prevent the need to rerun the experiments for such analyses. We will also share data and tissues with any other interested and appropriately experienced parties.

A retrospective assessment of reduction will be due by 22 November 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

As there is extensive understanding of the immune/inflammatory system the mouse, and as many genetically manipulated mouse strains available, the mouse is the species of choice for our studies. Many of the methods to be employed have been developed and refined over decades of immunology research and are therefore ideal for our intended purposes. Details of minimising animal suffering are given with each protocol.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The majority of our studies are carried out on adult mice as only in these animals will a fully developed immune and inflammatory response be mounted. There is extensive evidence that anaesthetics can alter inflammatory responses and thus our protocols cannot be carried out on terminally anaesthetised animals.The sentient response to the immune inflammatory response is also a key readout of animal well-being and thus the use of less sentiment animals is not appropriate for our studies. In addition, these animals will generally have an immune and inflammatory system that is less well developed than mammals and will therefore not be appropriate for the majority of analysis proposed.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Throughout, we will ensure that we apply the least invasive methods of dosing and sampling including the use of anaesthesia for humane restraint will carry out surgical procedures aseptically and with analgesia to control post-operative pain. To avoid multiple subcutaneous injections we will use osmotic mini pumps to minimise suffering whenever appropriate.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

We will follow practice guidance as outlined in the NC3Rs website.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

I sit on National grants panels and we have regular updates and discussions on 3Rs developments. We will also stay informed about further advances through the NC3Rs website. In addition there are numerous initiatives run by our REDACTED including local '3Rs' days which my group will attend to ensure that they are kept informed about the most up-to-date advances. We will also regularly access the REDACTED 3Rs website for additional up-to-date information.

Explain the choice of species and the related life stages

Over many decades, mice have been developed as the optimal species for studies of the immune and inflammatory response. Our ability to manipulate the genome of mice and the availability of a wide array of reagents capable of altering and manipulating immune and inflammatory response in mice further make them ideal for our study. In the main we will be using adult mice however we will occasionally study embryonic mice as we believe that some of the molecules that we study may be involved in helping protect the mouse during development.

A retrospective assessment of refinement will be due by 22 November 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

49. Toxicology of Chemicals

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)
- Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

No answer provided

Animal types	Life stages
Rats	adult, juvenile, neonate, pregnant, embryo
Rabbits	adult, pregnant, embryo
Mice	juvenile, adult, neonate, pregnant, embryo
Beagles	adult, juvenile
Pigs	adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The aim of the project is to evaluate the safety of various types of chemical materials when given to test animals. The work is designed to meet the requirements of government regulators in Europe and elsewhere, who must agree to the sale and use of chemical materials in society.

A retrospective assessment of these aims will be due by 11 December 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

The work is required to help protect the safety of people who are involved with the manufacture and transport of various types of materials, as well as those who may be exposed to them during deliberate use or accidental exposure. Without these studies, it is currently not legally possible to manufacture and sell these materials in the UK, in Europe, or into other markets in the world. Development and use of safer and better materials requires conduct of these studies.

What outputs do you think you will see at the end of this project?

Data collected will be information on the safety of various chemical materials, for those who would be involved in the manufacture, transport or use of such materials. This will include efforts to identify systems within the body or particular organs of the body that may be affected by short term exposure to, or accumulation of the chemicals. For example, blood testing and post mortem examination of tissues can demonstrate change in function or structure of body organs. Some studies will be to check if there is any effect on ability of animals to breed. This work then helps to predict effects that might be seen in people through exposure at work or everyday use.

The data will be collected to the standards required by government regulators in the UK, Europe and elsewhere, who will make decisions on whether these materials can be safely marketed and used in society.

Improved methods of conduct of specific data collection processes may be developed during the course of the project.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Our clients, typically commercial chemical companies, will benefit from the provision of high quality data. This will to help them in their work to produce safer and more effective materials which can be safely made and used without increasing health risks for those who make, use or transport them.

Enabling development of successful chemical materials will benefit society, for example by enabling improved crop yields.

The wider scientific community may benefit from publication of refined approaches to animal use.

How will you maximise the outputs of your work?

Our organisation has colleagues with extensive experience of such work in different parts of the world. Collaborations and information exchange with others within the organisation helps to identify and spread information on successful and unsuccessful approaches.

Collaboration with clients (knowledge gained on products).

On-going collaborations with NC3Rs on various aspects of regulatory safety studies, over many years.

Presenting outputs at scientific conferences and contributing to publications in the scientific literature where relevant.

Species and numbers of animals expected to be used

- Rabbits: 2600
- Mice: 9000
- Rats: 50000
- Beagles: 350

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Animals will be given a chemical material by the same method that people would be exposed to them - normally by mouth, or by inhalation. Inhalation of materials generally requires that animals are accustomed to close restraint in a purpose-made device while breathing the material. The materials will normally be given at a range of different dose levels, samples such as blood samples are commonly taken to assess any effect and/or to assess how much of the material is absorbed.
Behavioural tests may be conducted to check for effects. Some studies are to assess if the material has an effect on the unborn, or on the development of young animals. A small number of studies involve giving the material to rodents for an estimate of their lifetime, to check if the materials might cause cancer.

Animals will usually be used once only, and then will be humanely killed to check for effects in the body, including by examining the tissues microscopically.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

The process of dosing animals or taking samples can cause a degree of discomfort during conduct. Behaviour and health may be affected by the materials being given, and reduced health can be measured, eg by reduction in food consumption, weight loss, changes in blood results. Some studies may have effects on the ability to breed or on development of the young. In lifetime studies, adverse effects are usually those seen in ageing animals,

such as reducing function of the body's organs, resulting in reducing quality of life over time.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

The vast majority of harms described above are expected to fall within the mild category, some have no significant identified harm. Most animals of all species will have harms considered as mild or sub- threshold (not observed). An estimate of up to 15% of animals may experience harms categorised as moderate. Severe outcomes are not anticipated; if seen in individual animals, these would be reported to Home Office.

What will happen to the animals at the end of the study?

- Killed
- Rehomed
- Kept alive

A retrospective assessment of these predicted harms will be due by 11 December 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

While non-animal methods are used in some aspects of the programme of safety assessment of new materials, they are currently not able to predict effects on whole body systems or to provide information on how much of a material is absorbed. It is not currently possible to acquire all of the information on how the body systems such as the heart, liver and kidneys may be affected by new materials, without using animals. This information is essential, to protect people involved in the manufacture, transport or use of chemical materials. The protocols described in this project are conducted according to internationally-agreed guidelines, and are expected to be performed before government authorities will authorise the marketing of new chemical materials.

What was your strategy for searching for non-animal alternatives?

The organisation conducts non-animal tests as part of the programme of safety assessment of new chemical materials, but as noted, above, it is still considered essential by scientists and government regulators, to also do work using animals, which this project describes.

Why were they not suitable?

There currently remains general scientific agreement that to protect people involved in the manufacture, transport or use of chemical materials, non-animal alternatives do not, as yet, provide enough information to replace all animal studies.

A retrospective assessment of replacement will be due by 11 December 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimates are based on analysis of use of animals in an existing licence authorising work for the same purpose, combined with anticipated upcoming studies.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

The animal numbers for studies required by government regulators typically follow those identified in internationally-accepted guidelines, as expected to provide sufficiently significant outcomes. Longer term studies use larger groups as these are designed to enable identification of effects which might only be seen in only a small percentage of animals over a lifetime. Screening studies and dose ranging studies generally use minimal numbers, commonly between 1 and 3 animals per group, to generate initial data.

What other measures apart from good experimental design will you use to minimise numbers?

Pilot studies will be used to investigate the potential of new designs or processes to improve outcomes, before being used in larger numbers of animals. Initial screening studies, using small numbers of animals, are designed to identify and eliminate materials with undesirable results, and so reduce the numbers of animal which are then used in the studies required by government regulators.

A retrospective assessment of reduction will be due by 11 December 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Dosing of materials is by the same way in which people would be exposed during manufacture, transport or use of the materials, most commonly by mouth; occasionally by inhaling the material, or by applying to the skin. These are all very well established and common methods for the species to be used. Volumes of drugs to be given are in line with published guidance on minimising discomfort, and/or are known to cause minimal discomfort based on extensive experience at the site.

Blood sampling follows published guidance on suitable volumes which can be taken while minimising harms to animals.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The species used are selected based on known standards of outcome which will answer the scientific questions. They are species proposed as appropriate for the work by internationally-agreed guidelines, and are studies expected to be performed before government authorities will authorise the marketing of new chemical materials.

Response to tests is assessed over a time period which makes continued anaesthesia impractical, and would interfere with the outcome in most circumstances.

What are you going to do to refine the procedures (for example increased monitoring, post- operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Refinement of on-going procedures occurs as and when any concerns are identified; for example additional assessments may be included based on initial outcomes. Habituation of animals to restraint is a routine process, and the schedule can be amended in response to outcomes for individual animals.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

Dose volume and blood volume limits agreed with the animal welfare and ethical review body are based on the 2001 publication of Diehl et al: A good practice guide to the administration of substances and removal of blood, including routes and volumes.

Welfare end-points are developed in general line with publications on the topic, including the NC3Rs document from 2010 on dose level selection for regulatory toxicology studies.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Both our clients and our colleagues working in the same type of work in other countries, are collaborators who can bring ideas as to how to improve how to conduct our animal studies. Various staff at the establishment have been involved with working groups of the UK National Centre for the 3Rs (NC3Rs), over many years. Staff at the site routinely review published papers in the scientific press, some of which propose refined approaches to conduct of work.

Explain the choice of species and the related life stages

The animals (and age/life stage) used in the project have been shown to provide important information for assessing safety of chemical materials, and this is reflected by the inclusion of animal studies in European Directives and Regulations, and in internationally-agreed guidelines for how to best produce the safety information required.

A retrospective assessment of refinement will be due by 11 December 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?

Home Office

50. Tuberculosis pathogenesis & treatment in zebrafish and medaka

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

No answer provided

Animal types	Life stages
Zebra fish	embryo, neonate, juvenile, adult
Medaka	embryo, neonate, juvenile, adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our aim is to advance our understanding of how mycobacteria produce tuberculous disease and how the host responds to infection.

A retrospective assessment of these aims will be due by 04 August 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence? Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Our research is centered on understanding tuberculosis, a contagious infectious disease, caused by the bacterium Mycobacterium tuberculosis. This tuberculosis bacterium has very ancient origins and has been infecting humans at least for 30,000 years. Over the millennia, this highly specialized pathogen has evolved mechanisms to counteract, evade, and even benefit from host processes, particularly immune responses to cause disease. The tuberculosis (TB) bacterium has a complex life cycle in the host that involves distinct processes at each stage. As a result, from new infection to active disease, tuberculosis is delineated by complex steps, which remain largely unknown.

According to the most recent World Health Organization (WHO) report, TB caused disease in ~ 10 million individuals and killed 1.5 million individuals worldwide in 2018 (https://www.who.int/newsroom/fact-sheets/detail/tuberculosis). Additionally, tuberculosis has had important social implications for sufferers of this disease over the course of human history. TB sufferers are often not able to work or take care of themselves, and to make matters worse are stigmatized leading to social rejection and isolation even from family members.

The reason that TB continues to be an enormous problem reflects the failure of public health measures that have been implemented more affluent parts of the world - these include ventilation, improved air quality and nutrition. In the face of the inability of most of the world to implement such public measure, it is important to note that the currently available vaccine BCG and the currently available drug regimen to control the global burden have not been adequate to control the disease burden. This scenario is worsened with the emergence of multi drug resistant tuberculosis that is difficult to treat. The work described in this application aims to find completely new ways to treat and prevent TB through a better understanding of how it interacts with the host to produce disease.

What outputs do you think you will see at the end of this project?

This project is expected to yield new understanding of the bacterial and host determinants that promote tuberculosis infection and antibiotic resistance. In the next five years, we intend to continue to dissect and identify the genetic determinants responsible for the 47 susceptibility mutants identified during the previous license period. We also expect to find 50 additional mutants based on the number we identified in the last period. We will try to find drugs that can treat (counter) these susceptibilities, and expect to find hundreds of drugs based on our previous experience where the investigation of the mechanism of susceptibility of a single mutant has identified 15 drugs. We are currently also exploring the virulence mechanism of 5 bacterial determinants, and this will continue over the next five years. These studies will also lead to the identification of new drugs. Based on past experience, we anticipate identifying 5 new drugs from the study of these bacterial virulence mutants.

Who or what will benefit from these outputs, and how?

We expect that several of our findings will lead to human clinical studies and trials. During the last year, we have identified a new pathological pathway which led to the identification

of two groups of cheap, readily-available oral drugs that can be re-purposed to treat tuberculosis. We anticipate additional findings going to human clinical studies in this next phase of the project license.

Therefore, TB patients will ultimately benefit from these outputs. Since human studies and clinical trials take several years, this may not happen during the period of the project license. However, we expect that some human studies and clinical trials based on our findings will have started during the tenure of this license.

How will you look to maximise the outputs of this work?

We will disseminate our findings through publications and presentations at conferences and in various institutions. This will include the publication of unsuccessful strategies. We will continue to collaborate with human geneticists, with clinical tuberculosis researchers who can take the findings to human clinical studies, and with other zebrafish and mouse researchers, as warranted by the work.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 2,019,000 from egg to neonate; 1,011,000 from egg to adults
- Other fish: No answer provided

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We have developed the zebrafish and medaka as models to study tuberculosis by infecting it with the pathogen *Mycobacterium marinum*, a close relative of the human tuberculosis bacterium. *M. marinum* naturally infects zebrafish and medaka, causing a tuberculosis-like disease. This allows us to study tuberculosis pathogenesis in these organisms as the mechanisms of disease are conserved between these species of fish and humans. The zebrafish has proven to be an ideal model for the study of tuberculosis and has enabled us to address questions that have been elusive in the more traditional models of tuberculosis - mice, guinea pigs, rabbit and more recently nonhuman primates. The medaka has provided information into human resistance to tuberculosis. The model has several advantages over existing tuberculosis animal models which use the human tuberculosis bacterium in mice, guinea pigs, rabbits and nonhuman primates. First, because these fish naturally get tuberculosis with *M. marinum*, the disease is more similar to human tuberculosis than when the human tuberculosis bacterium is used in non-natural hosts. Second, the transparency of the fish larva allows for the direct visualization of the steps that lead to tuberculosis in live animals; third, the genetic tractability of the fish allows for the effective dissection of the host immunity to tuberculosis - genetic fish mutants that show variation in disease susceptibility can be identified and studied in detail. Fourth, the effective use of pharmacological interventions enables both identification of pathways in conjunction with the genetic studies above; it also helps identify new host- and bacterial-targeting drugs against tuberculosis. These unique benefits have together proved tremendously powerful and have allowed us to make surprising discoveries about tuberculosis pathogenesis that have immediate clinical implications.

We take advantage of the transparency of the the larval stage of the fish. The zebrafish and medaka are transparent during the first stages of their life, and this allows us to perform experiments that last only for a few days when possible. In the larva, we can visualize different stages of infection as they happen in real time in live animals.
For some experiments we use adult animals with a fully developed immune system to ensure that our results obtained in the larvae are reproduced in adults.

Typically, what will be done to an animal used in your project?

For genetically altered animal generation, maintenance, gamete recovery and breeding protocols:

Eggs might be injected with proteins, DNA or RNA to generate new genetically altered animal lines. In many cases, we attach fluorescent reporters that are light sensing substances to the DNA introduced into the animals. This allows us to monitor the introduced DNA under a microscope and allows the determination of the exact steps by which disease occurs. Because these experiments are completed before the fish are five days old, these are not regulated procedures. In case of new mutant lines, juveniles or adults will be genotyped by fin tissue removal under anesthesia at 2-3 months of age. Fish regenerate their fins, so these small amounts of tissue removed grow back within two weeks. Founder fish may have additional mutations, so they will be outcrossed with wild type animals to remove these mutations and establish the line that bears only the mutation of interest. Founders will be killed when the next generation has been genotyped. Animals from these new lines will be used to generate eggs by natural spawning. These eggs will be used in other protocols in the Project License . Some of the animals from these new lines may be used to recover gametes to cryopreserve the line (males) or to recover a line that has been cryopreserved (females). Gamete recovery will be performed in a given animal up to 5 times maximum and these animals will be used for natural breeding after this procedure. Animals used for breeding will be kept up to 2.5 years of age. Animals showing clinical signs of disease or damaged during maintenance and breeding procedures will be immediately killed.

For phenotyping of genetically altered animals with mutations causing harmful phenotypes:

Animals undergoing this protocol will be obtained from our genetically altered animal stocks by natural spawning. These animals might be screened by microscopy before five days of age and genotyped by fin tissue removal under anesthesia at 2-5 months of age when phenotyping experiments are performed blinded and the genotype is known only at the end of the experiment. All mutant animals will be killed immediately after showing clinical signs of disease.

For infection protocols:

For any given adult fish, bacteria will be administered by up to two of the described routes and if a subsequent drug/substance is administered, it may be by the same route or a different one with use of a maximum of 3 routes. Generally, for injected substances, we would expect to inject substances only once but there will be instances where a drug such as an antibiotic (e.g. streptomycin) has to be administered daily by intramuscular injection, as is done for human treatment. We will minimize the number of injections per animal. Bacteria will be administered to most adults only once with no further treatments. Survival experiments in adults will normally last for up to 2 months. All animals will be killed at the end of the protocol.

For any given larval fish, we will perform a maximum of three administrations of the infectious agent. We anticipate administering the compound at most once a day whether by soaking, injection or gavage. For compound administration, we will typically use only the immersion method unless the compound is not absorbed through immersion or we require its localization to a specific area. In rare cases, we may need to use up to 2 additional routes of administration. Bacteria will be administered to most zebrafish larvae only once. In many cases, these infected animals will be treated with substances by soaking which is not invasive. In some cases, other substances will be administered before or after infection by injection. Infection experiments in larval fish will be terminated when or before animals reach 14 days of age. All animals will be killed at the end of the protocol.

For fish younger than 30 days used for pilot experiments:

Animals undergoing this protocol will be obtained from our genetically altered animal stocks by natural spawning. These animals might be screened by microscopy up to five days of age. Substances will be administered to find the dosage used in further infection experiments. Pilot experiments in larval fish will be terminated when or before animals reach 14 days of age. All animals will be killed at the end of the protocol.

Use of non-Schedule 1 killing methods:

These methods will be used to kill the animals in experiments where biological samples or the whole animal must be preserved for future analysis. At any stage of the experiment and independent of the age of the animal, fish will be killed by anesthetic overdose (medaka and zebrafish) followed by either 1) Chemical dissociation of tissues (e.g. Phenol, Hot Shot buffer, detergent-based lysis buffers) to preserve genetic or protein material, 2) Chemical Fixation (e.g. Paraformaldehyde, Glutaraldehyde, (dithiobis [succinimidylpropionate]) or Dithio-bismaleimidoethane) to preserve structure of tissues and organs.

Only in exceptional cases where the anesthetic can interfere with instruments used for analysis, only zebrafish will be killed by use of ice slush (where the animal is physically separated from the ice) followed by snap freezing of fish in liquid nitrogen to preserve all components of the animals.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of infected fish will survive the injection, however it is anticipated that at most 5% of adults may die within 24 hours due to injection despite all attempts to identify these fish immediately after the procedure.

The infected adult fish will eventually suffer from the consequences of a tuberculosis-like disease manifesting lethargy and very small surface hemorrhages that are less than 1 mm. These clinical signs would increase in adult fish until they eventually become moribund and die from the infection, within weeks to months depending on the inoculum. Infected adult fish will be monitored twice daily and will be killed immediately when they show clinical signs of disease and before they become moribund, to decrease suffering. Despite in depth checking twice per day, there maybe up to 10% death with clinical signs such as lesions due to fish chasing (causing tearing of the wound) cannibalism and break down of tissues following death.

For larvae, 10% may die within 24 hours due to injection despite all attempts to identify these fish immediately after the procedure. Further, we cannot evaluate the severity of disease as they do not show any clinical signs of disease. However, to decrease suffering, infected larvae will be killed as soon as they become unresponsive to tactile stimuli (i.e. do not swim away when touched with a plastic pipette).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Protocol 1: 100% Mild
Protocol 2: 100% Mild
Protocol 3: 100% Mild
Protocol 4: 100% Mild
Protocol 5: 75% Mild and 15% Moderate, 10% severe
Protocol 6: 100% Mild
Protocol 7: 100% Mild

What will happen to animals at the end of this project?

- Kept alive
- Used in other projects

A retrospective assessment of these predicted harms will be due by 04 August 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Tuberculosis is a complex disease involving interactions between bacteria and many different types of host cells. Disease pathogenesis involves multiple cellular processes, including the movement of various types of host immune cells, engulfment of bacteria by these cells and the death of infected host cells and nearby cells. Moreover, multiple interactions between different host cells influence disease. This is exemplified in the tubercle, the hallmark structure of tuberculosis in which multiple host cells, both infected and uninfected, come together and form an organized structure in which the bacteria reside. The tubercle houses the tuberculosis bacteria and is the product of the interaction of multiple cell types. Adding to the complexity, tuberculosis can occur in multiple host organs and tissues. This multicellular structure is virtually impossible to replicate outside the host.

Which non-animal alternatives did you consider for use in this project?

In vitro infections using the various cells that become infected by the tuberculosis bacterium. We use these non-animal alternatives to ask very specific questions that are dependent only on direct interactions between the bacterium and its infected cell on its own, rather than in the context of the complex multicellular structure, the tubercle.

Why were they not suitable?

Human cells in culture do not always behave as they do within the tissues in the host.

A retrospective assessment of replacement will be due by 04 August 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Adults included in Protocol 6 are included in these numbers as they are the same animals (200,000 adult zebrafish and 10,000 adult medaka). We have estimated these numbers by the number of animals used under the previous Project License.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We use the NC3Rs experimental design assistant in combination with strategies to reduce the number of animals required for each experiment as described below.
We use pilot studies to look for differences caused by genetic mutations in the bacterium or the host, or by drugs that change bacterial or host determinants. This allows us to determine sample size for subsequent studies. For instance, a strong effect will allow us to reduce the number of animals used in subsequent studies.

Control animals are always required including for the pilot studies. When possible, we use siblings to minimize variability and thereby reduce sample size. Similarly, we use randomizations in all experiments: sibling animals from a single clutch are randomized into the various experimental groups. In the case of studies with mutant fish, we design our crosses so as to generate wildtype and mutants that are siblings. We perform genotyping only at the end of the experiment, so that we look for differences in infection without knowing which group the animal belongs to. This avoids any bias based on how our preconceived notion on how a mutation should affect infection.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will use PREPARE and ARRIVE guidelines at experiment planning stages and when preparing manuscripts for publication, respectively.

For all experiments, we use the minimum number of animals required based on prior experience. For new experiments, we run small pilot studies to assess the impact of a condition and how much it varies among the animals. This then allows to use statistical calculations to determine the minimum number of animals that we can use.
For experiments where the whole animal or tissues from the animal are preserved for further studies such us gene expression of tissue microscopical analysis, these are shared between researchers so the same experiment is not repeated while enough tissue sample remains.

A retrospective assessment of reduction will be due by 04 August 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use larvae, juveniles and adults of zebrafish and medaka. We use larvae rather than adult fish to the extent possible and limit the studies to a few days where possible. We are always seeking to refine our techniques to identify differences in the responses to infection to keep the infected animals for shorter time periods. This means that we can evaluate differences in the response to infection before the animals show any clinical signs of disease. We use adults for key confirmatory experiments only. All procedures in adults and larvae are performed on anaesthetized fish and animals are euthanized immediately once the experiment is completed.

Why can’t you use animals that are less sentient?

The bulk of our experiments involve fish larvae rather than adults. We do not use species such as fruit flies (Drosophila) and worms (*C. elegans*) that are used for certain types of research because these species do not have the same types of immune cells that humans do and that are important to fight against tuberculosis. Fish do have these cells and we have shown them to be similarly important in fish tuberculosis as they are in human tuberculosis.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

To minimize impacts in animal welfare during pairing to produce eggs, the spawning tanks are placed on shelves covered with a black background. This mimics the natural habitat where the light is entering the water from above and the bottom is dark. We have found this improves the number and quality of eggs that are produced. The tanks used to house the fish are transparent to allow the light enter the tank and the bottom is darker due to the shelving.

Genotyping trays are used to ensure fresh water and food is provided when the animals need to be individually housed. Fish are fed live diets using newly hatched brine shrimp (*Artemia salina*), which stimulates the animals as they chase the prey. Fish are housed in groups where possible, and are feed a suitable diet and follow a regime which has increased survival, development and breeding of the fish. When housing of three or two animals is required, artificial plant material is supplied to provide hiding places in order to decrease aggression. We use black shelving under each tank so as avoid stress from light surfaces and reflection.

Use of small molecule inhibitors or other chemicals (i.e. drugs) as a treatment for experimental groups: if the chemical has been used in the zebrafish or medaka before, we use that dose as a reference for the experiments under this Project License. In the case of new chemicals, pilot experiments will be used to test chemical toxicity. This will be done with small number of animals (5-10). The minimum nontoxic dose of chemical that has an effect in the outcome of the experiment will be used for further experiments.

All experiments will be ended early if it is determined that significant differences between treatments can be observed before the expected endpoint of the study. During all infection studies, any moribund animals (larvae or adults) are killed using a Schedule 1 method. For studies utilizing chemical or other synthetic compounds (e.g. drugs), we use the lowest effective dose that minimizes adverse effects.

Furthermore, in the context of severe severity work, all infected animals will be inspected twice daily as this will be enough to spot changes in behavior or clinical signs of infection before animals become moribund .

With respect to severity protocols, we do not feed the larvae for the 14 days post fertilization during which we conduct our experiments. We determined that this was advantageous to the health of the larvae under the conditions of the experiment where the fish water cannot be treated or replaced. The larvae derive all necessary nutrients from the ample high-density lipoproteins stored in their yolk. Restricting food from larvae from hatching to 14 days post-fertilization has been shown by our lab to reduce super infection of the larvae by food-borne commensal infection susceptibility.

Survival studies are a critical component of our goal to understand human tuberculosis. For example, host mortality can result from inappropriate immune responses rather than bacterial growth. In such cases, an analysis of bacterial burden may not provide understanding of a disease process. In these cases, survival studies can provide important insight into central disease processes. When survival studies are necessary, experimental animal numbers are minimized via statistical Power Analysis and up to 10% of animals undergoing this protocol may develop severe clinical signs of disease. To minimize suffering during these experiments, we kill any animal with clinical signs of disease or moribund immediately using a Schedule 1 method. All infected animals are meticulously inspected twice daily to ensure that moribund animals or animals showing clinical signs of disease are quickly detected in order to further minimize suffering. These checks are

conducted by experienced technicians and researchers who have had rigorous training and assessment before conducting checks alone. During these inspections, any action taken is recorded to be available for the researcher in charge of the experiment, the rest of the technicians doing the checks and the NACWO. Any fish found dead (<10%) or culled because of signs are recorded within the room and also a database. This allows all relevant parties to know the health status of the stocks under experiment and monitor numbers closely to ensure we work within the license allowances. In instances when unexpected number of animals show signs of disease, different from predicted by fish line and bacterial strain and inoculum used, the researcher in charge of the experiment, as well as the NACWO, is notified immediately.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Humane methods of euthanasia have been evaluated outside of the current and future acts of UK law relating to the use of animals in scientific procedures and have been cited as having a potential to improve the welfare of animals such as zebrafish RSPCA's 'Guidance on the Housing and Care of Zebrafish', May 2011. The method entails the immersion or exposure of zebrafish to temperatures of 4^{o}C or less for periods greater than 2 hours as a means to halt metabolism. Only 39% of chilled fish showed signs of distress, compared to 100% of fish exposed to the anesthetic agent MS-222 (tricaine).

After this step, the zebrafish will be subjected to Snap Freezing. The advantage of Rapid Cooling/Snap Freezing is to humanely euthanize zebrafish without causing them distress (Rapid Cooling/Snap Freezing as a means of euthanasia of ectothermic zebrafish for larvae up to 14 days of age. For euthanasia of adult zebrafish this method will require the zebrafish to be held within 2-4^{o}C ice slush until loss of operculum (the support structure on the side of a fish's head which forms a protective cover for the gills) movement followed by introduction to liquid nitrogen to confirm death and preserve tissues (snap freezing). We have received training to perform this procedure from the Zebrafish International Resource Centre and have since presented the method at meetings within the UK. Following this, other facilities have also adapted their protocols to incorporate this method due to its welfare improvement.

We will also use PREPARE and ARRIVE guidelines at experiment planning stages.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will be closely in contact with the NACWO, NIO and NVS to be informed of any new publications or reports regarding 3Rs. The NACWO will attend relevant continued professional development training as well as zebrafish husbandry meetings as with the previous license.

A retrospective assessment of refinement will be due by 04 August 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

51. Vaccines for arthropod-transmitted diseases

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Dengue, Zika, Chikungunya, Malaria, Vaccines

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Our research aims to develop and evaluate vaccines against neglected and emerging vector-borne diseases such as Yellow fever, Japanese Encephalitis, O'nyong-nyong, Dengue, Zika, Malaria, Mayaro and Chikungunya, which are overwhelming health systems in many countries. These diseases are all arboviruses transmitted to humans by *Culex sp., Aedes sp* or *Anopheles sp* mosquitoes present in tropical and sub-tropical countries and posing a threat to more than half of the world's population living in these regions. The difficult control of the mosquito transmission as well as the quick dissemination and increased risk in non-tropical geographical areas, due to global travel and trade, unplanned urbanization and climate change, makes our vaccine development research of great interest for the global health in endemic and non-endemic areas.

Our vaccines development benefit from the use of replication-deficient Viral-Vectored (VV) and Virus like Particles (VLPs) which are non-pathogenic and have been safely administered to humans in several clinical trials. We will develop novel and more efficacious vaccines by improving immunogenicity following several approaches: I. Test of wide variety of antigens, II. Use of clinical approved adjuvants to improve the

immunogenicity of the antigens, III. Comparison of different vaccine administration approaches to reach the infection site, IV. Combining different vaccines in single and/or multi-component platforms.

P. vivax parasite is one of the most widely distributed in the world and more difficult to eradicate from endemic regions. Although the mortality rate dropped in 2015 due to advances in malaria control, the current vaccines in clinical trials have failed in demonstrating a complete protective efficacy. One of our Malaria vaccines we developed will be used as part of our clinical trials and we have new candidates, which we aim to develop and evaluate immunogenicity, safety and efficacy during the course of this license (5 new candidates). Other less prevalent forms of malaria (*P.ovale, P. malariae, P knowlesi* and *P. cynomolgy*) are not considered a public-health emergency yet, but their importance is increasing since they occupy new niches available due to the decline in prevalence of their co-infections with *P. falciparum* and *P.vivax*. We aim to develop new vaccines against these four neglected parasites.

Dengue and Zika virus are flavivirus widely distributed in developing countries causing an important health threat for the World Health Organisation. The severity of Dengue virus has been associated to the subsequent exposition to different Dengue serotypes (with limited identity among them). We aim to develop a fully protective, universal efficacy against all the serotypes to eradicate mortality burden. Zika virus, in the other hand, has been linked to Guillain-Barré and neurological impairments in neonates. We have developed a vaccine (soon in a Phase I clinical trial) which has shown an induction of a long-lasting immunogenicity in pre-clinical settings. We aim to test the safety and efficacy during pregnancy to prevent vertical transmission and hence neurological diseases in neonates.

Yellow fever and Japanese encephalitis viruses are arbovirus causing thousands of deaths annually in South America and Asia respectively. The difficult production for the licensed vaccine for yellow fever virus as well as the difficulties in reducing adverse effects for the Japanese encephalitis virus vaccines, make the urgent need to explore our next generation vaccines. We aim to develop 2 new vaccines as immunogenic as the ones licensed.

Chikungunya, O'nyong-nyong and Mayaro viruses are alphavirus with a high mutation rate, making them more dangerous since they evolve to a more virulent strain. They co-circulate and are highly prevalent in Central and South America. Recently we have developed a new vaccine against Chikungunya which shows very promising results in a Phase I clinical trial. We aim to develop vaccines against O'nyong-nyong and Mayaro viruses which can be translated onto clinics.

Considering the overlapping incidence of arbovirus infection in many developing countries, we propose that combining different single vaccines or designing a multicomponent or multivalent vaccine which can target different arboviruses is of great interest because of the potential reduction in costs when administered in one shot.
Approximately 10 million people are infected with *Trypanosoma cruzi* worldwide and more than 99% of the cases occurring in the most impoverish regions of Latin America. We propose a target product profile for a therapeutic vaccine and then prevent (desired target) or delay (minimally acceptable target) the onset of Chagas cardiomyopathy and abdominal complications. We aim to evaluate immunogenicity of 3 new candidate vaccines.

In addition, our program of work with new vaccines and delivery methods, and new technologies such as understanding the genes or antigenic protein conformation involved

in successful vaccine-induced immune responses, may lead to novel knowledge that will significantly and lastingly improve vaccine development programs, efficacy, uptake and safety.

A retrospective assessment of these aims will be due by 27 July 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The data generated over the duration of this license will lead to journal publications, presentations at scientific meetings, organisation of conferences and symposia, patent applications, with leading vaccines progressing to manufacture for human clinical trials (short to medium term). Early clinical trials will be completed in the UK and vaccines showing good safety and immunogenicity profiles in UK trials will progress to clinical trials in countries affected by these mosquito-borne diseases, in particular targeting population in Mexico, Brazil, India and Thailand (medium-term benefit). The long-term aim is to develop vaccines that will in the future be included in worldwide human vaccination programs. Our ultimate objective is to prevent people from suffering and dying of these diseases. While this work will inform our vaccine developments and study designs, publication and presentation of this work will also inform other researchers and help to advance the overall knowledge in the vaccine development field. Currently, three of our vaccines (Zika, Chikungunya and Pv CSP-Malaria) are in different stages of clinical trials and we aim to add to the run at least two more in the following years.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We will use mice as they are the best characterized species for detailed immunological analysis as well as disease course. We will use approximately one thousand seven hundred mice per year, with a total of nine thousand four hundred mice along the five years of the license duration. Our project will mainly use wild type mice inbred (Balb/c, C57BL/6, NIH) or outbred (CD-1), in which a complete immune response will be expected and tested. However, some efficacy studies will be performed in genetically modified mice for example deficient in type I and/or type II IFN signatures, which are more susceptible to arbovirus diseases; or humanised chimeric mice to be able to mimic human immune responses.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

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

Most of our mice might experience discomfort after immunisation, but it will be minimized with the appropriate anaesthesia. A mild severity level will be expected. The administration of tolerated doses of vaccines, substances, cells or antibodies will be monitored because they might cause a systemic distress (<1%) such as piloerection and in the worst cases a monitored 15% weight loss. Frequent but small blood samples will be withdrawn. A moderate level of severity might be expected. A split-dose whole-body irradiation suffering will be minimised by providing supplemental nutrition and prophylactic antibiotics (NVS advice) and/or acidified water to prevent infection. Immune phenotype of irradiated mice will be reconstituted with progenitor stem cells of human origin to mimic human immune responses. A moderate level of severity is expected. Some mice will be aged to detect long-lasting immune response, but effects such as reduced function of vital organs, dental disease, spontaneous tumours… will be closely monitored and humanely killed to prevent severe illness. A moderate severity level is expected. As a results of malaria challenge, mice can develop systemic illness, but we will be monitoring them and will be sacrificed when parasitaemia becomes patent and before systemic severe illness develops. A moderate level of severity is expected. To maintain parasite development and production, mice used for mosquito feeding will be exposed to a mild severity level. In Dengue and Zika challenge, preliminary experiments will be set up to evaluate dose, the administration route and control the time points to avoid reaching severe illness. Evaluation of animal condition such as body weight loss, appearance and neurological signs will be monitored closely to prevent un-necessary animal suffering. In some animals (GAA) a severe neurological phenotype might be expected (ataxia/tremors) but humane clinical end-points will be set up and used to prevent excessive suffering. A severe level of severity is expected for the preliminary experiments but in following studies it will be reduced to moderate by refining the protocol. To evaluate vertical transmission of Zika virus, pregnant female mice will be used. Zika virus in GAA pregnant female mice induce placental injury and insufficiency, as well as high rate of foetal reabsorption and foetal brain injury. Preliminary experiments will be set up to evaluate the minimum dose which might show a subclinical phenotype in the parents but a viral transmission to the foetus to evaluate our vaccines. It is expected a severe level for the preliminary experiments but in the following experiments it will be reduced to a moderate level. Some mice will be assessed through a bio-imaging procedure and follow vaccine route and infection burden. Distress will be minimised by using appropriate depth of anaesthesia and good maintenance of body temperature. A moderate level of severity is expected. All rodents will be humanely killed for tissue assessment at the end of their experiments.

A retrospective assessment of these predicted harms will be due by 27 July 2025

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

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

One of the components of vaccine development is to demonstrate immunogenicity, efficacy, safety and potency in animal models. No alternative to animal research has been developed to date, but the use of a low number of animal species is of high consideration. Vaccines requires the demonstration of their therapeutic or preventive potential before any possible translation into clinics and therefore an approval release for a clinical trial. Although many *in vitro* (such as test of ADE, Neutralisation assay, antigen expression…) and in silico research allow us to optimize a good vaccine candidate for animal testing, it cannot mimic and predict the complexity of the whole immune system.

A retrospective assessment of replacement will be due by 27 July 2025

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

The group sizes are reduced and optimized by power calculations. Power calculations are performed in order to use the smallest number of animals needed to provide satisfactory analysis of data.

Many experiments need the inclusion of control groups such unvaccinated animals or un-related vaccines. To minimize the use of control groups, we will maximize their use by combining several hypotheses in a single experiment.

Along the evaluation of a time course, multiple sampling (tail-bleeds) per mouse will be performed instead of using different sets of mice per time-point. Some immune responses can be tested with very small volume of blood.
At the end-point, we maximize every experiment by harvesting and freezing multiple tissues for an “a posteriori” use in different assays. In this scenario, we avoid using more mice to answer the different questions arisen.

To limit biased interpretation of the treatments and ensure robust findings we will use a good experimental design with blinding and randomisation.

A retrospective assessment of reduction will be due by 27 July 2025

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Mice are the most characterized species for detailed immunological analysis as well as disease course. Mice have been proved to be excellent indicators of immunogenicity enabling the clear assessment of novel vaccines and vaccinations regimens for improvements.

We will use the most refine method of administration of substances unless shown to be inefficacious. We aim to develop vaccines to be needle free such as biojectors or reduce the administration regimen by using encapsulated vaccines which provide a continuous and sustained release after administration. Temperature through the implanting of microchips and body weight measurement will be evaluated closely to avoid unnecessary distress as well as micro-sampling for blood withdrawal to test blood parameters that reflects disease and illness. A behavioural assessment will allow us to identify the onset of neurological illness and therefore early humane-end points and severe clinical illness. The reduction of adjuvants used, since they can enhance immune response and consequent adverse effects, is one of our primary strategies in vaccine development. We aim to develop a mathematical prediction formula for disease development by using variables such as temperature, viremia, RNA levels…etc, and predict when clinical disease will happen and avoid suffering.

A retrospective assessment of refinement will be due by 27 July 2025

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind?
- During the project, how did you minimise harm to the animals?