



Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project
licences granted July – September 2024
that require a retrospective assessment



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1. Preclinical development and assessment of medical interventions against antimicrobial resistant bacterial pathogens

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

Microbiology, Vaccine, Antibiotic Resistance, Intervention, Countermeasure

Animal types	Life stages
Mice	juvenile, adult, aged
Guinea pigs	juvenile, adult, aged
Rabbits	juvenile, adult, aged
Hamsters (Syrian) (Mesocricetus auratus)	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.

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 overall aim of this project is to develop and/or assess new vaccines, therapies, and treatments (interventions) in animal models of infection against several bacterial infections associated with antimicrobial resistance. These infections are considered to be a public health threat in the UK and globally, for which current countermeasures are absent, inadequate, or unproven.



A retrospective assessment of these aims will be due by 2 January 2030

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?

This project will allow for the use of established animal models as well as furthering their development. These models will enable studies into the development of disease and transmission of bacteria impacting public health. They will also support the development and approval of vaccines and treatments.

The data generated will enable the selection of potential vaccines and treatments. This will benefit humans as antimicrobial-resistant bacterial pathogens have the ability to harm or even kill large numbers of people with underlying health conditions including, but not limited to, cystic fibrosis and Chronic Obstructive Pulmonary Disease (COPD).

We will also improve the data available to scientists about how animal models respond to specific bacterial pathogens and the vaccines and treatments that treat them. This will benefit humans because in the future, vaccines and/or treatments will be selected with a growing likelihood of success.

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

There are several benefits of this project in developing models for and evaluating the efficacy of treatments for bacterial pathogens.

Outputs will include:

- Model development and optimisation in a range of species; new information on the models will lead to publication and dissemination of refinement information to with wider bacterial pathogen and animal community
- Pre-clinical evaluation of novel vaccines and treatments against bacterial pathogens to assist in development and licencing.
- Capability to assess vaccines and treatments against emerging bacterial pathogens, where human clinical trials are not possible.

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

This project will contribute to the understanding and refinement of animal models of bacterial pathogens allowing long term contribution to the field. We anticipate that model development and optimisation in a range of species can be achieved within this project. Knowledge and findings will be transferred to subsequent studies and disseminated to the scientific community through publications where appropriate. New techniques and findings



will also be disseminated to the wider community.

This project will enable us to select effective vaccines and medicines from a range of candidates. By filtering these candidates through our models of infection, we will reduce the number of candidates required to be tested in humans and advance translational research.

This project will provide the capability to assess new interventions against the most serious forms of infectious disease, where human trials will not be possible due to the severity of the form of disease being investigated. Regulatory bodies will, however, accept preclinical data generated in animals in such circumstances so this project may assist in the licensing of new or improved interventions against serious forms of infectious disease.

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

Our work is highly collaborative, with the assessment of potential new vaccines and medicines being carried out with either commercial partner or through research programmes.

Knowledge and findings will be transferred to subsequent studies and disseminated through the scientific community through publications where appropriate to ensure that our findings are subject to the scrutiny of the scientific community. In addition to peer-reviewed publications, the work performed under this licence will be disseminated widely at international conferences which would provide an opportunity for informal feedback and in-depth discussions to disseminate new knowledge. The data provided to collaborators and customers will direct the appropriate generation of products which will have direct benefits to human health.

Species and numbers of animals expected to be used

- Mice: 4000
- Guinea pigs: 1250
- Rabbits: 1250
- Hamsters (Syrian) (*Mesocricetus auratus*): 750

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.

Mice, hamsters, guinea pigs and rabbits are all recognised animal models for the assessment of the bacterial pathogens mentioned within this project. Mice, hamsters, guinea pigs and rabbits will be used to evaluate the effectiveness of vaccines and medicines against bacterial pathogens. The scientific community working on these bacterial pathogens worldwide use the same animals as these are the most appropriate species in these disease investigations. Using the same animals allows for data that is generated from this project to be compared to other related work that has been published. This is an important and critical step in the development and assessment of various vaccines and medicines.



Although the majority of animals on this licence will be adults, there may be a need to develop animal models of infection in juvenile and aged animals, as some pathogens documented within this licence have been shown to have greater severity in humans, within these age groups.

The use of two or more species is required by licencing authorities to provide reliable data, which fully assesses new vaccines and interventions candidates. Adult, juvenile, and aged animals for each of these species are most suitable for these experiments.

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

Typically, the following will occur during and experiment:

Following acclimatisation, animals will be sedated and have a small amount of blood taken, to assess normal immune responses prior to intervention. Animals on this project may also be implanted with a biometric chip or other telemetric device to provide a unique identification code and/or to track body temperature or other data.

Animals may then be vaccinated, by routes similar to those used in people, such as into the muscle or up the nose. Alternatively, medicines may also be administered, most commonly orally.

If a vaccine is being assessed the duration of the experiment can range from one week to several months depending on the length of the vaccination phase.

A bacterial pathogen or combination of bacterial pathogens will be administered to animals, usually via the aerosol route and allowing the animal to breathe in the pathogen. Once administered the experiment is usually completed within 28 days, depending on pathogen.

These animals will undergo regular health monitoring by trained and expert animal care staff at specific timed points. The frequency of these health monitoring checks may be increased as signs of disease present in these animals. If clinical signs approach the severity defined within the project the animals will be humanely killed to relieve their suffering by an approved method.

Animals can be expected to be sedated and sampled (nasal washed/swabbed) daily in order to study the shedding of the bacterial pathogen administered. Animals may be sedated and have a small amount of blood collected over the duration of a study. Ensuring that published recommended guidelines for blood withdrawal are never exceeded.

At the end of all studies, animals will be killed humanely by an approved method. After being humanely killed, samples of organs, tissues or relevant biological material will be collected for analysis.

The majority of animals on this licence will be manually handled, with or without anaesthesia, in order to inject or give them vaccines or therapies against specific bacterial infections; or to check their overall health and weight.

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

Potential adverse effects to pathogen challenge, anaesthetics and administration of substances have been identified.



Animals may be manually handled, with or without anaesthesia, this process may cause short term distress to the animals. Animals may also be implanted with a biometric chip or other telemetric device, typically under their skin. This is expected to cause no more than mild and transient discomfort.

Some of the experimental drugs may cause harm for example by disrupting the normal community of beneficial bacteria in the gut. Respiratory infection in animals is likely to develop very quickly and may overwhelm the animal due to rapid septicaemia. The likely adverse effects will include a period of fever, a combination of clinical signs of infection associated with the infectious agent, including weight loss, changes in breathing rate and lethargy. If clinical signs include signs of imminent death, the animals will be humanely killed by an approved method.

Regular health monitoring will be conducted by trained and expert staff members to detect clinical signs of disease. If clinical signs are present, the frequency of these health monitoring checks can be increased, but in some cases, animals may die before such signs are evident. Previous experience in similar bacterial pathogens indicates that although this can happen, most our procedures will be moderate in severity.

Septicaemia is expected to occur within a small percentage of animals (approximately 10%). The bacterial pathogens named within this licence are expected to have a range of expected mortalities due to the nature of the disease they cause. Bacterial septicaemia can cause death within a very short time frame. In such a scenario, it is unlikely that there will have been a significant period of 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 animal type)?

Model development

The expected severity from bacterial pathogen infection is expected to be severe in more than 50% of mice, hamsters, guinea pigs and rabbits on this project

Immunogenicity

The expected severity from vaccine administration is expected to be moderate in 10% of mice, hamster, guinea pigs and rabbits on this project

Pharmacokinetics

The expected severity from therapeutic/antibiotic administration is expected to be moderate in 20% of mice hamster, and guinea pigs on this project

Protection Testing

The expected severity from protection testing against bacterial infection is expected to be severe in less than 40% of mice, hamster, guinea pigs and rabbits on this project unless protection is observed.

Refined Model development



The expected severity from bacterial pathogen infection is expected to be moderate in more than 60% of mice, hamster, guinea pigs and rabbits on this project

Humane clinical endpoints have been clearly defined therefore unnecessary suffering is avoided. At the end of all studies, animals will be euthanised by a Schedule 1 method or by terminal exsanguination under full anaesthesia.

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

- Killed

A retrospective assessment of these predicted harms will be due by 2 January 2030

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 research focuses on assessing the effectiveness of treatments for infections caused by bacterial pathogens. There is a requirement to develop new treatments and vaccines for a variety of reasons; either current treatments have adverse effects, a lack of evidence of efficacy or are untested against new or emerging health threats.

The way in which the human airway protects themselves against bacterial pathogens and the way in which the immune system works is complex and has not successfully and reproducibly been modelled in computers or cell-based systems. We cannot show whether a vaccine or treatment can both reduce symptoms and prevent progression to disease in the body except by using animals.

Suitable and appropriate animal models must be utilised to fully assess the immunological response to a treatment or vaccine within a biological system. These animal species are the most appropriate models for the bacterial pathogens assessed within this license, as they have similar respiratory and immunological responses to that of humans. Currently there are no alternative or substitutive methods which could be utilised to fully achieve our aims. However, constant monitoring of publications on new technologies and improvements to cell based and computer models will constantly be assessed and where appropriate non-animal models will be used.

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

As much evaluation of the efficacy of new treatments as possible will be done using in vitro systems, such as Minimum Inhibitory Concentration (MIC), Minimum Bacterial Concentration (MBC), Minimum Biofilm Eradication Concentration (MBEC) and cell-based assays of biological activity. In addition, insect models (*Galleria mellonella*) of infection



have been developed for some infectious organisms and have been used as a first "in-life" model system to help filter through large numbers of potentially useful antibiotics. In certain cases, the number of drugs required to be tested in our mammalian models may be reduced by replacing the initial drug screens required. Drugs that fail in these special cases will not be taken forward.

We will, however, try to use human sera, wherever possible to assess human vaccines for biological activity. In addition, although insect models of infection are available, the relatively simple nature of these models makes them only suitable for early stage high throughput screening. However, if insect models suggest a therapy or intervention may show promise, mammalian studies of efficacy will need to be conducted to assure regulators that the candidate is effective in more complex animal models.

Why were they not suitable?

To establish reliable evidence of efficacy of new treatments which show promise using in vitro testing or insect models of infection, it is still essential to assess them using the full range of host-pathogen- treatment interactions which mammalian model systems offer. Regulatory bodies will accept robust efficacy data derived from relevant animal models of infection, where classical human clinical trials cannot be performed, to support license applications. At present, there are no alternative in vitro technologies that replace the need to use animals since there is a requirement for all components of an immune response must be present within the systems used to investigate novel new vaccines, therapies and treatments (Interventions), to better predict the effect of that potential new treatment in man.

A retrospective assessment of replacement will be due by 2 January 2030

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?

Over the next five years, it is possible that a variety of species are required to develop models of infections and to assess the properties of new interventions. Although it is very difficult to predict how many treatments we will test during the life of this project, our past performance indicates that we will use at least 1000 animals each year. The level of usage may, however, increase in any year due to the possibility that a candidate vaccine or therapy which may suddenly require a lot more testing, as it enters clinical or other critical studies. As a result, we predict to use the following: Mice 4000, Hamster 1250, Guinea pigs 1250, Rabbit 750.



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

The organisation has a vast amount of knowledge and experience when designing studies, with the aim of using the number of animals per group while still generating data that are useful. Statistical advice is available, and this advice will be used to minimise animal usage in studies.

We always use statistical calculations to identify and use the minimum number of animals required on a study, whilst still providing robust scientific data which will be accepted and give statistical relevance and comply with any relevant regulatory requirements.

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 required for a study will be determined by how variable the infection model outcome is. We reduce our model variability by optimising our infection procedures using good microbiology and world class instrumentation.

Studies will be conducted in a step-wise manner so that the number of animals used will be minimised if the vaccine/treatment shows no likelihood of working; for example, if a new vaccine does not elicit an appropriate immune response, then it would not progress to a challenge efficacy study.

Where appropriate, pilot studies with a reduced number of animals or groups, will be performed. This is particularly important where challenge dose must be determined, or to assess vaccines ability to elicit an immune response or to establish a dosing regimen. Although statistical significance would not be determined, in the long term, this avoids a negative outcome involving large number of animals.

Robust scientific quality control of the test materials and methods will ensure studies are carried out successfully the first time, minimising the need to repeat studies and subsequently reduce the number of animals used. All protocol and study designs would be reviewed by the organisations ethical review panel and improvements and suggestions would be implemented where possible.

A retrospective assessment of reduction will be due by 2 January 2030

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 majority of our work will be conducted in mice. Mouse models are very useful as they are widely used by the scientific community because there is such a large body of information already published about mouse biology. Sometimes, however, mouse models are not adequate or not as closely matched to human disease in which case we need to use other species.

To assist in the prompt recognition and subsequent intervention, critical periods will have been identified (depending upon pathogen) and monitoring frequency increased. Our staff work in shift patterns, to ensure that animals considered to be in the critical phase of an experiment are regularly monitored. This high frequency, hands-on monitoring, has been shown to effectively minimise the welfare costs to animals and hence reduce the severity and quickly identify if an animal is heading towards a humane end point. Humane clinical endpoints have been clearly defined therefore unnecessary suffering is avoided.

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

The majority of animals used within this project will be mice. The mouse model is an established model for vaccine studies. Less sentient species have immune systems which are too different from humans. The use of other species would not fully represent the complex immune system response to these vaccines and therapies, which could provide a poorer indication of the likely responses if this was to progress to human clinical trial. The use of two or more species is required by licencing authorities to provide reliable data, which fully assess new vaccines and interventions candidates

Additionally, there is a large body of published research which enables a direct comparison of the effects of particular vaccines in mice and humans. As immune responses typically can take weeks to develop a response to infection, vaccine or therapies, it is unfeasible to keep animals under anaesthetic for that long.

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

We strive to conduct the minimum number of experiments as possible, to collect data to exemplify the interventions associated within this licence. We use rigorous monitoring processes to ensure that we minimise the welfare costs to the animals. Our staff work in shift patterns to ensure that animals considered to be in a critical phase of an experiment are regularly monitored. The frequency of which can be increased whenever necessary depending on the severity of the presenting symptoms. The health scoring system we have developed over many years provides a more holistic overview of the potential harms we are causing and when to intervene such that harms are minimised. The high frequency of hands on monitoring has been shown to effectively minimise the welfare costs to the animals.

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

We will follow a variety of published guidelines where appropriate, including:

Local AWERB guidelines



ARRIVE guidelines of the NC3Rs

guidance on Animal Testing and Research from the Home Office

Good research practice guidelines from the Wellcome Trust

LASA and RSPCA guidelines and

Handbook of Laboratory Animal Management and Welfare, Forth Edition Editor(s): Sarah Wolfensohn, Maggie Lloyd. Published Online: 04 Jan 2013, Print ISBN: 9780470655498.

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

All staff working under this project attend relevant training and refresher courses 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. Any changes that will be beneficial to the animals while maintaining the scientific integrity of the protocols will be investigated and implemented where appropriate. Changes will be made to this project, if required, to implement advances in the 3Rs effectively.

A retrospective assessment of refinement will be due by 2 January 2030

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. Improving vaccine immunogenicity

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Vaccine, Virus, Immunity, Lymphocytes, Cancer

Animal types	Life stages
Mice	adult, embryo, neonate, 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.

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?

This project aims to develop methods of improving the performance of vaccines in order to induce more comprehensive and long-lasting protection.

A retrospective assessment of these aims will be due by 1 January 2030

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?

Findings of this project will directly inform the design of better vaccines against influenza virus. The new knowledge gained from the study will also be applicable to the design of vaccines against other infectious agents and cancer.

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

This study will generate 1) new information regarding how the immune response can be stimulated, 2) publications detailing this work to be shared with the scientific community and the public and 3) the potential for new vaccine development through commercial partnerships.

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

White blood cells called T-cells are crucial for generating an immune response to fight infection. In the case of certain infections such as influenza virus, these T cells often fail to offer complete protection against serious illness due to the ability of the virus to change key characteristics which enable it to hide from the immune response. We hypothesise that by making T cell responses stronger, we will endow them with the power to withstand these changes and offer protection against multiple strains of influenza. The World Health Organization (WHO) estimates that flu is responsible for the deaths of between 250,000 and 500,000 people around the world every year.

Indeed, influenza epidemics, in the UK alone, currently result in hospitalisation of thousands of people annually resulting in death in approximately 600 individuals thus better vaccines are urgently required (<http://vk.ovg.ox.ac.uk/vk/influenza-flu>). We are also constantly at risk of the emergence of radically altered influenza viruses (pandemic strains) with the potential to claim tens of thousands of lives if effective vaccines are not put in place. We have generated a wealth of data already which points to a new approach by which influenza vaccines can be improved and we have strong signals which indicate that increasing the strength of influenza-specific T cell responses improves immunity to different influenza viruses. In this current study, we will build on this work to generate and test improved vaccines against seasonal and pandemic flu.

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

Several international collaborators are already involved in this work who are experts in their fields.

New knowledge, including importantly unsuccessful approaches, will be shared via our collaborators' networks, meetings and conferences, and through publication. We will also share our research with the public. Examples of the activities we have been involved in include:

1. Co-producing a "science and philosophy" project for primary school children with a member of our faculty.
2. Co-producing an "Art in Science" project with a school teacher in a local high school.
3. Radio and television interviews.
4. Coffee shop and "Pint of Science" talks.



5. Speaking at public events organised by the home institution.
6. Development of a flip-book to explain organoid technology to the public.
7. Production of a computer game designed to educate players in the relationship between the immune system and cancer. This exciting new initiative will enable players to understand the complexity of barriers to “beating” cancer, including the nature of the vessels through which cancer cells and immune cells move from one site to another.

We will continue to seek exciting opportunities to develop our resources and skills in this area e.g. a senior lab member recently attended an NC3Rs course to learn how to communicate information on the use of animals in research in educational establishments (schools and colleges).

Species and numbers of animals expected to be used

- Mice: Mice: 3500

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 study how effective our new vaccines are in mice. The advantages of using mice are: 1) their immune system resembles that of humans, 2) we have many tools to study different aspects of the immune system in mice that we cannot apply to other systems and 3) vaccination has been studied in mice for a long time and results have been translated to improve human health successfully.

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

We expect to use approximately 3500 genetically modified mice. Approximately 1500 of these will be vaccinated and around half of these will also be challenged with influenza virus.

Vaccination: an animal will be restrained for around 30 seconds for the delivery of a vaccine via an intra-nasal route or an intravenous, intramuscular or intra-peritoneal injection. Discomfort is minimised by good training techniques. Animals may be briefly anaesthetised for this procedure in order to ensure that the vaccination is successful. In some cases, a second vaccination will occur in the same way to boost the immune response.

Infection: an animal will be restrained for less than 30 seconds for the intra-nasal delivery of virus. Animals will be briefly anaesthetised for this procedure in order to ensure that the virus infection is successful. Without anaesthetic, animals will typically sneeze thereby expelling the virus.

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



Genetically modified mice will be bred for this study. A minority of the animals will be immunodeficient and may, in principle be more infection-prone. However, as a result of good housing conditions, we do expect that mice bred for this study will be healthy and will not suffer from spontaneous infections or inflammatory diseases. Most mice will be vaccinated with the experimental vaccines, designed in the course of the study. These vaccines are modified versions of previous vaccines, differing mainly in their ability to induce immune responses to influenza virus. These responses are not expected to cause harm to the animal. In the case where mice are infected with influenza virus, it is likely that some of these, in particular unvaccinated mice, will develop typical symptoms of viral infection. These include listlessness, loss of appetite and weight loss. Animals will be monitored daily during this period and provided with alternative dietary products. In the unexpected event that recovery is unlikely, animals will be humanely killed.

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)?

Vaccination: severity will not exceed moderate for approximately 80% of mice. Of the 20% which experience >15% weight loss, all mice are expected to recover.

Influenza infection: due to weight loss of greater than 15%, approximately 50% of unvaccinated animals are expected to fall into the severe category. For mice whose weight drops below 20% following infection, 50% are expected to recover weight above the 20% threshold and whereas 50% are expected to be culled following failure to recover.

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 1 January 2030

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?

Experiments to test vaccine immunogenicity cannot be conducted in vitro as the extent of cell activation is determined by a number of integrated signals including antigen processing, presentation, growth factor production and T cell frequencies. Spatiotemporal considerations also influence the outcome of antigen presenting cell - T-cell encounter. Thus, studies such as this can only be conducted in the context of a fully integrated immune system. Moreover, this study will also test vaccine efficacy.



To achieve this it is important that we are able to assess protective immunity in the context of in vivo disease models of virus infection.

The mouse provides an excellent model in which to study the immune system. Mice are well characterised immunologically, and their immune systems closely resemble those of humans. In addition, a very large number of studies have previously utilised mice to study interactions between influenza virus and the mouse immune system. Thus, a large number of optimised reagents exist to facilitate designs of robust and reproducible experiments.

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

We are developing non-animal alternatives. We have recently been awarded an NC3Rs grant to develop human organoid cultures in order that we may examine interactions between the human immune system and colorectal cancer. Using similar technology and working in collaboration with an expert, we are developing tonsillar organoids which may allow us to study the interactions between virus infected epithelial cells and human immune cells. Using this system it may become possible to test novel vaccines using human tissue and cells.

Why were they not suitable?

The tonsil organoid system is under development and is not yet proven as a method for testing vaccine performance. We will be introducing the system during the early stages of this project whereby the mouse work will help us validate the models' performance. Clearly, we will look to replace the use of mice as soon as this becomes feasible.

A retrospective assessment of replacement will be due by 1 January 2030

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?

Experimental designs to be utilised are simple and have been used, with slight variations, in our laboratory for a number of years. As such there will be little optimisation of regimes required, keeping the number of animal required as low as possible. Animal numbers are also minimised by the use of good statistical tests. The aim of this research is to identify immunological effects that are both reproducible and unambiguous. Using these criteria and depending on the precise nature of the experimental question, minimum group sizes of 5 - 9 mice will provide interpretable statistically significant results. Experiments will also be performed on three separate occasions.



New laboratory based methods, which have become readily available within the last couple of years, allow for extensive analyses of small numbers of cells. Within the last year we have upgraded our equipment to more sensitive/more parameter flow cytometers and advanced techniques and equipment for histology. This allows us to gain a great deal of information from organs recovered from a single mouse. This has the advantage of enabling us to gain a more comprehensive view of a given immune response but also significantly reduces the number of animals required to answer most of our experimental questions.

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

The lab routinely uses the NC3Rs Experimental Design Assistant to perform and refine sample size calculations as more experimental information is gained. Expert statistical support is available within our laboratory and professional statisticians are available through the Research Institute, in which the lab is based. As well as scheduled one-to-one meetings, these statisticians offer weekly drop-in data clinics to discuss experimental design and data analysis with all members of the Institute.

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

Experimental work is coordinated between different members of the laboratory and breeding is overseen by a laboratory technician who has oversight of all projects. This ensures that breeding is conducted with precise numbers in mind in order to ensure minimum wastage and maximum sharing of tissues.

Pilot studies will be carried out to test novel vaccines. Statistical test are not applied as the experiments are carried out in order to detect relevant "signals" such as successful vaccine delivery. A positive signal will lead to a powered experiment whereas a negative signal will lead to the vaccine being discarded.

A retrospective assessment of reduction will be due by 1 January 2030

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 mouse provides an excellent model in which to study the relationship between the



immune system and virus infection, since mice are well characterised immunologically, and their immune systems closely resemble those of humans. In addition, we will make use of genetically modified mice which enable us to study T-cell responses to the same peptide/MHC complexes as in humans (HLA-DR1 transgenic mice). These mice provide an ideal opportunity to perform detailed studies of anti-viral T cell responses using vaccine strategies and peptide modifications directly relevant to humans. There is a large body of data using mouse models to test the efficacy of vaccines derived from viruses that are later used in humans.

We have studied anti-influenza immunity using mouse models for around 16 years. During this time several refinements have been made which minimise mouse numbers. A major improvement is to deliver influenza virus to mice under anaesthetic. This results in more uniform dosing of mice and hence less variability in the type and magnitude of the immune response induced.

In all procedures animal suffering will be minimised through good animal handling techniques and strict adherence to monitoring procedures outlined in detail in Section E. Lethal challenges with influenza virus will not be utilised during the course of this study as the laboratory is well equipped to assess influenza virus titres thus induction of protective immunity will not be assessed through survival but through comparisons of virus loads in unvaccinated versus vaccinated animals. In addition, humane endpoints have been clearly identified. Animals infected with influenza virus are weighed daily and animals losing more than 25% bodyweight are killed. This is a rare event, as sub-lethal doses of influenza virus will be utilised.

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

These studies require an evaluation of how the immune system responds to vaccination and / or infection with influenza virus. This involves the coordinated activities of many different cell types which behave differently according to which tissue in the body is affected. A key feature of a successful vaccine is the ability to induce long-term immunity, protecting the host against exposure to an infection which may occur many months after vaccination, therefore this process can take weeks to several months.

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

We have been working with mouse models of influenza virus for approximately 20 years thus we are well versed in recognising and mitigating against adverse effects of infection. Several scientists in the lab are expert animal handlers and have worked in the animal house at the home institute for over 10 years. Training is provided to all new starters and experts guidance and advice is always on hand.

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

We will use NC3Rs tools and the ARRIVE guidelines to ensure our experiments are conducted in the best possible way.

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



We stay informed about advances in the 3Rs by attending 3Rs workshops and meetings. We take every opportunity to implement approaches which could reduce animal numbers. One such approach which is currently under development in our lab, is the use of tonsil organoids to examine interactions between influenza infected cells and the immune system.

A retrospective assessment of refinement will be due by 1 January 2030

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. Inflammation in parasitic worm infection and allergic 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

Inflammation, Immunity, Parasitic infection, Allergy

Animal types	Life stages
Mice	embryo, neonate, pregnant, 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?

The overarching aim of our research is to determine which cells are involved, and which mechanisms and pathways are used, to initiate, maintain and regulate a form of inflammation, called 'Type 2', a distinctive immune response found mainly in parasitic worm (helminth) infections and during allergic responses. These studies will not only provide fundamental understanding of the requirements for an efficient Type 2 immune response, but should also provide proof of principle data for future therapeutic development.

A retrospective assessment of these aims will be due by 3 January 2030



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 global need to cope with parasitic worm (helminth) infections and allergies. Helminth infections affect a third of the world's human population, causing widespread suffering and, in some cases, lethal disease, while allergies are at epidemic levels in the developed world, and an increasing concern around the globe. This project aims to help our understanding of these conditions, as well as their relationship to one another. Given the well-established role of environmental, non-harmful microbes (such as gut bacteria) in modulating immune responses, part of this project will involve investigating whether such microbes contribute to regulation of inflammation during helminth infection or against allergens. Our ultimate goal is identification of cellular and molecular targets for rational development of novel therapeutics, which is particularly urgent for helminth infections, for which there are currently very few effective treatments, as well as concerns about emerging drug resistance.

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

This project aims to disseminate new knowledge by publication in peer reviewed journals and presentations at conferences, seminars and workshops. We hope that in the longer-term our work will contribute to new immunology-based therapies for helminth infection and allergies. In the 5 years of this project, we aim to continue our high standard of publication, averaging more than 5 research papers per year in highly-respected peer-reviewed journals.

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

This project aims to answer basic scientific questions. The new knowledge generated, and the unravelling of important fundamental immune mechanisms, will be relevant to a broad range of human and animal conditions, including helminth infection and allergies.

In the short term, the primary benefit of our work will be to discover new knowledge about fundamental mechanisms that control the initiation, maintenance and regulation of Type 2 inflammation.

In the longer term, this will ultimately provide novel candidates for the future development of innovative therapeutics targeting cells or their products (in any inflammatory disease). It also has the potential to direct drug development.

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

Communication of our findings will be primarily through publication in widely-read peer-reviewed journals, but also presentation at local, national and international congresses and



institute seminars. To ensure maximum dissemination, only journals that allow open access without payment by the reader will be considered. To prevent unnecessary repetition of experiments by others, we will seek to publish all data generated under this project, including negative results.

To enable rapid translation of our findings to the clinic we will exploit new and existing collaborations, and with local clinicians as part of the translational environment within our institution. We have highly effective systems in place for technology transfer. Additionally, we aim to expand our current collaborations with pharmaceutical and biotech companies by presentation at national and international forums at which industrial representatives are present.

Species and numbers of animals expected to be used

- Mice: 12,500 for protocol 1 (breeding) and 13,000 for experimental protocols.

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 study mice because the immune system, tissue organisation and development of all mammals are similar, allowing mice to be a model for humans and other animals. We also use mice because scientists have created many genetically altered mouse lines that allow us to dissect in fine detail what happens during immune responses, including in helminth infection and in allergies. Genetically altered mice, and many of the tools designed to work with mice, allow us to define in precise detail how particular cells and molecules of the immune system work together to fight helminth infection or cause allergic responses. By manipulating these cells and molecules, we can identify the immune components that are central to Type 2 inflammation, and use that information to help design future therapies.

We will generally use adult mice in our experiments (>6-8 weeks of age), as at this age the mouse's immune system has developed to a degree that models the adult human immune system. However, important data suggest that modulation of the immune system in early life can alter susceptibility to external insults such as infection. Therefore, in some experiments we will use juvenile mice to try and uncover important ways in which alterations in the immune system during early life can have important effects on the immune system.

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

Typically, mice will be infected with helminths (e.g. via the skin, which is the natural route of infection, or by injection) and/or be exposed to an allergen (e.g. via inhalation, or by injection), and/or receive a single or multiple injections containing immunomodulatory substances (e.g. antibodies to neutralise a specific immune mediator or deplete a specific cell type, or cells to promote a particular response).

Experiments might look at the immediate immune response (e.g. by analysing blood samples, or immune cells isolated from organs such as the lung) in the first few days after helminth infection, allergen exposure, or administration of an immunomodulatory



substance, or may last several months to allow full response development, or assessment of immune memory. These experiments will typically last between 1 day and 3 months. For longer experiments, mice may receive multiple doses of an immunomodulatory substance (e.g. once a week for 2-3 weeks), and samples (e.g. blood) may be taken to monitor immune response development over time. Experiments will end with animals being killed humanely, sometimes under terminal anaesthesia.

The cumulative experience of mice will typically be exposure to 2 or 3 procedures that may each cause short but usually separated periods of typically mild or potentially moderate degrees of suffering.

Separate from the above experiments, some genetically altered animals will be used only to breed and maintain animal lines.

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

Most of the animals undergoing experimental procedures, even with immunological manipulation, will experience mild, and no more than moderate, severity limits.

In most cases, only a small proportion of experimental animals will develop beyond mild symptoms. Breeding and maintenance of genetically altered mice are not expected to exhibit any harmful phenotype. Our assessment of the importance of immune cells during inflammation generated by the body in response to challenge with substances can result in local or systemic inflammation and pathology. This can manifest as weight loss, the involuntary bristling of fur, reduced spontaneous activity and reduced response to external stimuli. However, in most cases, only a small proportion of experimental animals will develop beyond mild symptoms. Helminth infected mice may show swollen and distended abdomens. Some helminth infected animals can suddenly die from week 5 onwards, with no overt warning signs or evidence of suffering (a feature of infection that is also found in human disease). However, the majority of our experiments will not involve strains of mice, doses or times post-infection that will lead to this level of severity. In investigating lung inflammation, some animals (5- 10%) may experience temporary (less than 24 hours) minor laboured breathing. The investigation of established models of intestinal inflammation generally result in symptoms including weight loss, inactivity and loss of appetite, which can sometimes lead to bleeding and prolapse. Although dietary manipulation will typically be well tolerated without adverse effects, in some cases mice may exhibit marked weight gain, moderate weight loss, or failure to gain weight over the baseline. Some protocols will involve general procedures such as restraint, injection or use of anaesthesia. All of these provide the possibility of adverse effects, but none beyond moderate severity. All animals will be humanely killed at the end of each 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 animal type)?

This project works to a maximum severity of severe. However, across the license, we expect the majority of mice to experience mild (approximately 40%), sub-threshold (approximately 20%), or moderate (approximately 40%) severity, with a minority of animals (<0.5%) that die suddenly, without evidence of suffering beyond moderate severity limits,



being recorded as severe.

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 3 January 2030

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 mammalian immune system is highly complex, relying on the co-ordinated actions of multiple different cell types and molecules that collectively provide protection. Additionally, tissue environments can play a major role in modifying immune responses. As such, the insight that animal experiments can provide on the mechanisms necessary for Type 2 immunity is of significant relevance.

Unfortunately, experiments in a laboratory dish or test tube (*in vitro*) are unable to reflect the cellular and molecular complexity of the immune system and tissue environments in the whole body.

Therefore, the use of mammals is essential for gaining a better understanding of the mechanisms underlying Type 2 immunity.

Mice will be used in these studies because their immune system closely resembles the human immune system therefore giving a better chance for translating results for future therapies. Additionally, a wide array of wild type and genetically altered strains of mice are available that will allow us to better decipher the role of immune cells and molecules in Type 2 immunity. Finally, a vast range of reagents is available for analysing murine cellular and molecular interactions during immune responses.

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

- 1) Use of cell lines and *in vitro* systems.
- 2) Analysis of human samples.

Where possible, we use *in vitro* systems to address specific questions. For example, when we identify the induction of particular proteins in a particular cell type in our animal experiments, we see if we can replicate this *in vitro* using related cell lines and the mediators observed in the animals. If we can replicate a specific aspect of our data, detailed analysis of signalling pathways is undertaken *in vitro*.



Why were they not suitable?

Our studies rely on looking at the immune response to infection or other conditions in the context of the whole body. In particular, our research aims to identify key cellular and molecular interactions processes during inflammation in tissues such as the lung, liver, intestines and female reproductive tract. Unfortunately, it is not possible to emulate these tissues and processes *in vitro*. Many location- specific features of immune cells are lost once they are removed from tissues, which makes the use of cell lines impractical. *In vitro* systems typically allow for the study of one or two cell types in a highly controlled environment that is not reflective of the complex immune system in the whole body. Therefore, to fully understand how different cell types and molecules co-ordinate an effective immune response we require animal experiments.

Experiments to track immune cell interaction, activation and function in tissues are not possible with human samples (which are often restricted to peripheral blood). Nor can we experimentally manipulate humans.

We will regularly review the literature regarding *in vitro* approaches with human and murine samples (such as precision cut tissue sections), and alternative animal approaches (such as zebrafish systems) so that, as promising new approaches are developed, we can test them and exploit them if possible.

Our research also depends on generating the egg life cycle stage of the helminths we study for use in subsequent studies. There is no alternative means of generating this stage other than in a mammalian host (usually mice).

A retrospective assessment of replacement will be due by 3 January 2030

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 mice has been estimated based on experience gained under my previous Home Office licenses, taking into account breeding strategies for genetically altered mice, and anticipated numbers of planned studies over the course of the license.

Reflecting Replacement, Reduction and Refinement of our protocols over the past 5 years, we have reduced our anticipated numbers of animals for this application by approximately 35% from our previous PPL (39,700 > 25,500).

What steps did you take during the experimental design phase to reduce the number



of animals being used in this project?

For all of our experiments, in-bred mice are used to reduce experimental variation, which makes it possible to use fewer animals to achieve statistical significance. For the majority of our studies, mice from the same litters are used for control and experimental mice, reducing variation that can occur due to differences in microorganisms (e.g. gut bacteria). Overall, our experiments are designed to reduce the number of variables (for example age) to as few as possible and thereby reduce the number of control groups required.

We work with our Establishment 3Rs Manager to ensure all lab members are introduced to the NC3Rs experimental design assistant and encouraged to use it. Everyone in the lab is trained in statistical methods and these are regularly discussed at lab meeting, to ensure all agree the best methods are being used. This includes animal randomisation (to reduce unintentional bias in selecting animals, that could influence results), and blinding (to reduce unintentional bias in analysis, by keeping the identity of animals/samples hidden from the researcher until the end of the experiment), whenever practically possible. Tissue-sharing is a major tool we use to reduce animal usage.

A significant proportion of our animal use is related to breeding programmes for genetically altered lines. We follow the advice of our animal facility staff to optimise breeding, and regularly discuss numbers at lab meeting to ensure we do not overbreed. Where possible and appropriate, we use substances that can target or block immune processes in wild type mice, to reduce use of genetically altered mice.

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

We routinely perform pilot experiments to determine the optimal number of mice to achieve statistical power. Experiments are then performed on a minimum of two separate occasions to ensure reproducibility, following which data pooled from experiments are statistically analysed to reveal less pronounced effects without increasing overall animal use.

We have many years of experience in planning animal experiments and we plan our research to ensure that all animals are used most effectively. We often combine experiments to ensure that multiple organs are used to address multiple objectives at once. Careful discussion between multiple researchers is required to avoid compromises. Due to high variability in immunological and infection models *in vivo*, especially in genetically altered models that we have yet to assess, we will adjust groups sizes as required, should subsequent power calculations indicate that this is necessary.

The increased use of genetically altered animals has led to more complicated breeding strategies and, as a result, larger colonies. We reduce the numbers of these animals in our experiments by using littermates as controls wherever possible. Additionally, when a particular strain is not being used experimentally we work closely with the animal technicians to develop a breeding strategy that maintains low numbers of stock animals (applying concepts from the ASRU GAA mouse breeding framework).

In many experiments, we will use bone marrow to carry out pilot studies *in vitro*, therefore only a few animals are used to initially test new hypotheses. Importantly, we continue to work closely with collaborators who can supply bone marrow samples, therefore reducing the number of genetically altered animals that have to be bred to facilitate experiments.



A retrospective assessment of reduction will be due by 3 January 2030

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 mouse models to study Type 2 inflammation. Mice represent the most appropriate species for animal study of immunity, because of the extensive knowledge of their physiology as it relates to humans, the genetic and biological tools available and the ability to be easily bred and handled.

The helminth we study, *Schistosoma mansoni* in mice is the most established model for human schistosomiasis. Parasite migration, maturation, egg production, and pathological consequences of infection in the mouse are similar to the processes in humans. A proportion of *S. mansoni* infected mice may show signs associated with disease (abdominal swelling) from approximately 6 weeks post infection. Doses are carefully adjusted to minimise adverse effects.

Sensitisation and challenge of mice with allergens or allergen-loaded cells generates airway inflammation that is an accepted model for studying mechanisms underlying human asthma. Similarly, mouse models of intestinal inflammation are the foundation of innovative research into colitis and inflammatory bowel disease in humans.

Most of the animals undergoing experimental procedures, even with immunological manipulation, will experience mild, and no more than moderate, severity limits. Doses and timing are carefully managed such that animals will experience minimal suffering. We are constantly assessing and refining our approaches to ensure robust experimental results whilst minimising pain, suffering or distress.

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

To our knowledge, no other species of lesser sentience can fulfil the requirements of this project to the same extent as the laboratory mouse. We are studying long and complex immune processes, and trying to understand how different cells communicate to orchestrate an appropriate response in Type 2 inflammation. Although animals such as *Drosophila* and zebrafish do have an immune system, this is a lot less complex than the mammalian immune system and so cannot respond in the same way to the infectious and inflammatory systems that we study (e.g. helminth infection, or allergen exposure).



Additionally, *S. mansoni* cannot be used to infect these less sentient animals.

We cannot use terminally anaesthetised animals as we require the mice to develop immune responses to the external challenges, and to analyse the outcomes of these challenges over time and in response to interventions.

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

All procedures will be performed by trained and skilled personal licence holders, who will handle animals with care. Animals will be monitored for adverse effects using score sheets previously developed in conjunction with the NVS and NACWO. These score sheets have proven to allow for objective measurements of clinical signs associated with adverse effects to determine when humane endpoints have been reached.

In line with the establishment's policy, we will adopt the latest techniques in animal handling (e.g. cupping) to significantly reduce the stress associated with procedures. Furthermore, where possible, the least invasive methods for dosing and sampling will be applied.

Anaesthesia and analgesia will be provided where suitable (e.g. for humane restraint, during or in recovery from surgery).

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

We will follow:

The government animal testing and research: guidance for the regulated community (<https://www.gov.uk/guidance/research-and-testing-using-animals>)

Morton et al 2001, Refining procedures for the administration of substances; Laboratory Animals, 35, 1-41

The NC3Rs webpage: <https://www.nc3rs.org.uk/>

Standard Operating Procedures developed with the animal facility and the named veterinary surgeon.

For specific models, we read papers from other groups doing similar experiments, as well as consulting directly with other researchers to discuss the most refined procedures.

We will continue to use the NC3Rs Experimental Design Assistant to ensure we design experiments that will allow us to achieve statistical significance whilst minimising the number of animals we need to use.

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

Our animal facility includes a team of dedicated veterinarians that are continually seeking to improve animal welfare and refine animal use. We consult closely with them and take full advantage of the extensive resources provided to ensure we are following current best



practices. We will continue to work closely with our local 3Rs representative to ensure we stay informed about the advances in the 3Rs. For example, we attend Experimental Design workshops to ensure we achieve our scientific aims with the minimal number of mice.

A retrospective assessment of refinement will be due by 3 January 2030

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?



4. Influenza infection in murine models

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

genetics, antiviral, vaccine, influenza, immunity

Animal types	Life stages
Mice	adult, embryo, 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.

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 overarching aims of this project are to understand the effects of Influenza A virus on the mammalian body in both the long- and the short-term and to devise and test strategies to intervene and mitigate such effects.

A retrospective assessment of these aims will be due by 1 January 2030

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?

Influenza virus infections are a significant global health concern, causing widespread illness and death, with seasonal infections responsible for hundreds of thousands of deaths annually. Pandemics can occur when novel strains of the virus emerge and infect humans, swine, or avian populations. Given the endemic infection of wild bird populations with highly pathogenic strains, the virus represents an ongoing threat to both human health and agriculture. The high mutation rate of the virus allows it to cross species barriers, despite fundamental differences between hosts, for example base temperature. Understanding how these adaptations occur is of significant interest to scientists to determine how species jumps may occur, for example the jump of an avian virus to a mammal host.

The influenza A virus has a high mutation rate, while the host genome changes slowly. The interplay between the genetic backgrounds of both the virus and the host influences the host immune response, sometimes leading to severe illness and viral symptoms. The interplay between the viral and host genomes occurs on many levels. Influenza A virus genomes capture substantial genetic diversity, allowing the viruses to evade host immunity and produce viral factors that interfere with its regulation. On the host side, host gene expression is regulated not only by genetic sequences but also by associated structural and chemical modifications of the DNA molecule such as methylation. Researchers aim to dissect the mechanisms underlying the relationship between viral and host genomes and investigate the effects of severe infection in the long and short term to develop methods to mitigate against severe infection.

Vaccination is the preferred strategy to prevent influenza infection or reduce its associated pathology. However, the high mutation rate of the viral genome means that available vaccines do not provide long-lasting protection, we are in an ongoing 'arms race'. In the face of novel pandemic strains, the delay in the production of new vaccines may allow significant spread of the virus. Antivirals, such as Tamiflu and Relenza, can provide prophylactic protection and are somewhat effective in the treatment of influenza virus infections. However, the ease with which the virus mutates means that resistance to antivirals that target virus gene-driven processes can rapidly develop (e.g., H1N1 strains circulating prior to 2009 were resistant to Tamiflu).

Pregnancy represents a major risk factor for severe viral infection, but the effect of maternal cytokines and chemokines on the developing fetus during such an infection is unknown. Maternal exposure to virus infection may alter genomic imprinting of fetal DNA, despite the fetus not directly experiencing infection. This has been studied to a limited extent for bacterial and yeast infections, but the consequences of maternally-restricted viral infection remain unexplored.

In summary, there is a need to:

1. Identify novel genetic components of the host-pathogen interaction in mammalian systems infected with influenza A virus.
2. Develop novel antivirals as a first line of defense against pandemic influenza and to treat severe infections in susceptible individuals.
3. Develop new vaccine strategies that can lead to expedited vaccine production.



4. Determine the potential long-term effects of influenza A infection in infected individuals and their progeny.

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

The primary benefit of this research will be new insights into the genetic mechanisms that determine how the mammalian host interacts with an influenza virus on a molecular level, particularly in mice. The findings will shed light on the initial immune responses triggered by influenza A viruses in mammals, how they could influence future responses upon subsequent exposure, and the role of genetics in this process. Additionally, the research will help to better understand the pathogenicity and virulence of evolving influenza A viruses.

Additionally, this work proposes novel methods for influenza vaccine strain production through the development of attenuated viruses that can enhance the host's response to infection. Other benefits will include the identification of new mutations within the influenza A genome that can alter the host's response and studying how maternally restricted viral infection during pregnancy can affect embryonic signaling molecules and the fertility of resulting offspring.

The team will be trained in the necessary protocols for the research, and they will present their findings at scientific conferences and publish peer-reviewed articles on their work.

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

Short-term outputs: The academic community will benefit from the data and methods generated under this license, which will be made available to scientists within and outside our establishment to facilitate collaborative work. Additionally, peer-reviewed research publications, conference abstracts, posters and presentations, and internal and external seminar presentations will inform the research community and stimulate further research. Specifically, the license will generate novel data in the fields of influenza A virus vaccine development, anti-influenza drug development, genetics underlying the host- pathogen interaction, and understanding of the long-term effects of respiratory pathogens on directly infected individuals and their progeny.

Long-term outputs: Novel research in in vivo control of respiratory pathogens may result in intellectual property that can provide a competitive advantage for research and commercial activity in the UK. The license also creates the potential for collaboration with industry partners to further develop treatments for short and long-term illnesses resulting from respiratory pathogens. The license will also provide career development opportunities for early career researchers through training opportunities.

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

These data will be presented at national and international conferences, and published in peer-reviewed academic journals. Currently, we are working towards at least three publications that are in review/preprints that include data generated from influenza infections in mice. We expect these outputs to be realised within the time frame of this licence.

Protocols developed as part of this work will be made available via peer-reviewed



publications. Prior to publication, our methods will be shared informally with collaborators to ensure maximum utility of our field-leading protocols. Publications of methods will include details of approaches that were unsuccessful/ sub-optimal, to minimise the likelihood of repetition by other groups.

Additionally, data we generate will be used by members of our group and our collaborators (approximately a half dozen members of staff/ students at our institution per year) to support academic activity that additional to inclusion in manuscripts will be included in doctoral theses and grant/ fellowship applications.

Species and numbers of animals expected to be used

- Mice: 2400

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.

Our studies are aimed at understanding the genetic mechanisms underlying interaction between virus and the host, in particular the role of the immune response (as dictated by the host genetic background, or elicited by the viral genetic background) in viral pathogenesis, and in testing the efficacy of prophylactic and therapeutic agents that will modulate viral pathogenesis during influenza infection. Gene expression is cell-type specific, and therefore the heterogeneous cell population within a host organism includes many cell types, each with a unique expression profile that is affected by cell-cell interactions, and changes during the immune response. The simultaneous contributions from different cell types interacting with each other, within the context of the lung and/or uterus is of sufficient complexity that it cannot yet be replicated or reproduced faithfully in vitro.

Murine models have been used for many years to model the host-pathogen interaction between influenza A virus and the mammalian immune response. Mice therefore are currently the most thoroughly understood model organism for influenza infection.

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

The animals will be lightly anaesthetised to allow the intra-nasal administration of influenza A virus, and/or treatments that will modulate the resulting infection. They will then recover from anaesthesia and will be monitored for any adverse responses to the treatment. The course of infection will be closely monitored. Where necessary for experimental aims, further steps will be taken before or after viral administration. These may include the insertion of temperature monitors to allow for close monitoring of less obvious clinical signs, or timed matings. The animals will typically be killed by schedule 1 methods at the end of these experiments to allow us to examine tissues post-mortem.

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

Influenza Infection (Protocols 2 and 4)



Infection of mice with influenza A virus is potentially lethal. The categories of 'severe', 'moderate' and 'mild' infection are well defined and have been developed with input from the literature, local researchers, vets and facility staff over many years.

First signs of infection are likely to be ruffled coat and hunched appearance within 1-2 days post-infection, and progressive weight loss; ultimately infection may result in respiratory distress, extreme lethargy, coma and death. High doses may cause death in 2-3 days, though this varies with the virus strain and mouse line used and this is a rare occurrence which we work to avoid in highly controlled experiments. In some cases, doses of the virus that cause clinical signs 3-5 days post-infection (severe infection) may be required to meet scientific aims. Where it is possible to achieve scientific aims with a moderate infection, pilot experiments will be carried out to identify moderate doses that do not typically cause severe infections, as described. In many cases, valuable data can be obtained from animals that do not exhibit clinical signs, that is, early in infection or with a low to moderate dose of virus.

Mice undergoing experiments to look at the early stages of infection do not develop severe clinical signs as the experimental end-point is reached prior to severe disease onset. However, studies designed to look at the long-term effects of infection may require that animals develop moderate or severe infections and then recover. In this case, our aim is to establish a virulent infection that will generate a considerable immune response, but the infection will not necessitate humane culling. In these experiments, 90% of the infected mice would be expected to develop substantial symptoms, not reaching a humane endpoint, with recovery from infection within 14 days.

Fever is a common immunological response to pathogens in many mammals. It is possible that some strains of particular virulent viruses have evolved to benefit from the hyperthermic immune response. Alteration of environmental temperature to within the murine thermoneutral zone (30C) is unlikely to cause discomfort. It is more likely that mice will be more comfortable at this temperature than their usual hypothermic conditions. Mice may become pyrexemic when infected with influenza A. Upon infection, animals will be monitored and their temperature recorded twice per day. Palatable food mash is also provided within the cage to encourage feeding and aid in hydration. Additional clinical signs that will be monitored and recorded will include significant loss of condition, agitation, unresponsive to stimuli, significant wetting around the snout or excessive panting. Internal temperature will be monitored. Any mouse who exceeds 40C after infection will be culled by schedule 1 methods. Cage condition will also be closely monitored to help reduce the number of environmental stressors on the animals.

Mice will be treated with synthetically engineered non-naturally occurring vaccine-candidate viruses (i.e., live attenuated vaccines/LAVs), with the specific and sole intention of examining the use of such agents as immune-modulating substances. LAV-treated mice will be monitored as described for influenza A virus infections above. Every effort will be made to determine a titre for in vivo use that leads to no or mild/moderate clinical symptoms. Not all in vivo effects can be predicted.

Pregnant mice are likely to have a more severe response to influenza A virus infection due to the immunomodulatory effect of pregnancy. All influenza A virus titres will be tested prior to the main study in a small cohort (usually 2-4 animals) to ensure that only a moderate infection results. The results from these pilot studies will be added to the final studies. Moderate infection will be limited to a clinical score >5 using the clinical score method



outlined in the appendix. Weight changes will not be used to assess clinical severity, as these will be masked by an undetermined number of developing fetuses. Early fetal loss and resorption will not be detectable using non-invasive methods. Spontaneous late-term abortion is an unlikely consequence of severe infection based on information from the current literature.

Dose finding for putative treatments (Protocol 3)

Every effort will be made to determine a range of doses that are safe for in vivo use prior to any studies being carried out. However, not all in vivo effects can be predicted from published data or in vitro experiments. All experimental mice will be monitored closely and any animal that develops clinical signs (up to 5% weight loss for 72hrs, loss of condition, moderate piloerection, reduced spontaneous activity and poor response to stimuli) will be humanely culled by schedule 1 methods or exsanguinated under general anaesthesia.

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)?

The study will involve several protocols using adult mice. Breeding and maintenance is expected to be sub-threshold.

Of mice infected with influenza A virus, we expect 50% of them to exhibit moderate symptoms, 20% to exhibit mild symptoms, 25% to exhibit sub-threshold symptoms and <5% to exhibit severe symptoms.

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 1 January 2030

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 aims to understand the genetic mechanisms underlying the influenza A virus infection in a mammalian model in both the long- and the short-term, and to devise and test strategies to intervene and mitigate such effects. Thus, our studies are aimed at understanding the interaction between the virus, the host, and in some cases the progeny of the host. In particular, we will study the role of the immune response, as dictated by host



and/or viral genetic background, in viral pathogenesis and test the efficacy of prophylactic and therapeutic agents that will modulate viral pathogenesis during influenza infection.

As gene expression is cell-type specific, each cell type has a unique expression profile that is affected by cell-cell interactions and a plethora of biological chemicals generated as part of the immune response. The simultaneous contributions from different cell types interacting with each other, within the context of the lung and/or uterus is of sufficient complexity that it cannot yet be replicated or reproduced faithfully in vitro.

Wherever possible, virus replication and the effect (on- and off-target) of potential antiviral agents will be characterised in vitro, or using ex vivo materials, prior to the infection of mice. Results obtained in vitro will be used to inform and design our in vivo experiments. However, a number of factors including in vivo toxicity, metabolic processes, and mode of delivery mean that agents that are effective in vitro may not be effective within an infected host and therefore efficacy must be determined within an animal model.

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

In silico/bioinformatic techniques, such as meta-analysis of publicly available data, have been used to collate existing published and lab-generated data to determine gene targets and therapeutic targets/targets for prophylaxis during influenza infection.

Wherever possible, virus replication and the effect of potential antiviral agents will be characterised in vitro, or using ex vivo materials, prior to the infection of mice. Results obtained in vitro will be used to inform, design and optimise in vivo studies.

Why were they not suitable?

As gene expression is cell-type specific, each cell-type has a unique expression profile that is affected by cell-cell interactions and the immune response to infection. The simultaneous contributions from different cell types interacting with each other, within the context of the lung and/or uterus is of sufficient complexity that it cannot yet be replicated or reproduced faithfully in vitro. Although great strides have been made to replicate such interactions in silico, a faithful replication has not been established.

In the context of prophylactic development, a number of factors including in vivo toxicity, metabolic processes and mode of delivery mean that agents that are effective in vitro may not be effective within an infected host and therefore efficacy must be determined within an animal model.

A retrospective assessment of replacement will be due by 1 January 2030

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 have been estimated based on the numbers used over the course of the previous licence and more than 8 years expertise, and includes animals bred and maintained as part of the previous licence.

For infection studies, we can generate useful data from small, usually $n = 5$ per group, studies since there is typically a clear & measurable distinction between control/untreated animals.

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

In silico/bioinformatic techniques, such as meta-analysis of publicly available data, have been used to collate existing published and lab-generated data to determine gene targets and therapeutic targets/targets for prophylaxis during influenza infection.

Wherever possible, virus replication and the effect of potential antiviral agents will be characterised in vitro, or using ex vivo materials, prior to the infection of mice. Results obtained in vitro will be used to inform, design and optimise our in vivo studies.

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

We perform pilot studies to validate new protocols on small numbers of animals to make sure that any protocol is well tolerated and to minimise the risk of unintended adverse effects. Pilot studies are designed in such a way that they can augment larger studies that follow to avoid repetition.

The majority of our studies are performed in a staggered manner where we observe the outcome of small groups of animals and monitor for unexpected outcomes before completing the rest of the group with the same protocol. Where appropriate, tissue from control animals is used to pilot subsequent studies in vitro/ex vivo.

Breeding practices will be of optimal efficiency, with mouse stocks maintained at minimum numbers required to maintain the colony.

A retrospective assessment of reduction will be due by 1 January 2030

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.

Mice are readily infected with appropriate strains of influenza which cause similar pathogenesis to that found in other species susceptible to influenza infections, such as humans and pigs. Infection of mice with influenza can cause severe symptoms. In our experience, the trajectory of clinical signs follows a defined curve, though this may differ between strains of virus and strains of mice. We use, as much as possible, the minimum dose of virus that gives statistically interpretable results and, where possible, to implement an experimental design that includes culling of animals prior to the appearance of clinical signs. Where the minimum dose is unknown, we will perform a dose-response study and implement a staggered approach to the dosage.

In the past, we have observed mild severity in 71% of cases which includes: short duration of piloerection, weight loss of <10 percent, and altered ambulation. Roughly 1% of mice display severe signs, based on the numbers in the previous iteration of this licence. Viruses will be administered using intranasal inoculation, a mild procedure with short recovery time that is closely representative of the way in which influenza infections are acquired in nature.

Temperature monitoring will allow us to, in some studies, observe the effects of the virus in vivo prior to the onset of clinical signs. Additionally, although the murine response is representative of the human response to influenza, some stark differences remain. It is possible that the hypothermic reaction to the virus is a primary cause of this difference. Manipulation of environmental temperature may allow us to more specifically replicate the immune and physiological responses seen in human influenza infections in this model. Such a situation can provide a more robust, specific and refined model for studying influenza infection.

Drug treatments will usually be administered using bronchoalveolar lavage. This allows the most standardised delivery of drug dose amounts to the correct body system that would be targeted for treatment if drugs tested were to be used as prophylactics or therapeutics. Alternatively, intravenous or intramuscular administration may be applied if this mimics the approach most likely to be taken in the clinical setting. The discomfort caused by these routes of administration are short in duration.

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

Influenza A virus strains are host-specific and most strains infect only live mammals and avian species. The aim of this project is to understand the host response to Influenza A virus in a mammalian system, specifically mice as a proxy for larger mammals including pigs and humans.

Replicating the multicellular processes that are pivotal to the progression of Influenza A infection necessitates a whole animal. Where possible, we do use cell culture, air-liquid interfaces, co-culturing of different cell types and ex vivo tissue that maintains cellular composition and architecture.



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

Each study will be reviewed by our named vets to ensure it is the best approach to achieve the scientific aims. All efforts will be made to ensure studies are defined as mild or moderate. Severe protocols will only be considered if necessary to achieve scientific aims. Severity for each study is pre-determined based on available data - that is, a mild or moderate study can be carried out on a severe protocol.

Animals will be monitored daily. If any animal is approaching the severity predetermined for that study monitoring will increase to twice daily.

We will attend conferences, read relevant literature, and discuss protocols with colleagues (both internal and external) who work in a similar field to allow for the continuous refinement of protocols.

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

We will make use of the resources at the NC3Rs website <https://nc3rs.org.uk/who-we-are/3rs> and the NC3Rs regular newsletters to keep up with advancing knowledge and technology.

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

Attending conferences, reading relevant literature, and discussing protocols with colleagues (both internal and external) who work in a similar field will help increase awareness of advances that allow for the refinement of protocols.

The regular local review of study request forms by our named vets and the in-house interaction with our animal technicians is also valuable in continually refining our protocols. We also receive regular updates from the Named people within the establishment about updated best practices.

A retrospective assessment of refinement will be due by 1 January 2030

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. Diagnosis of Statutory and Endemic Avian Viral 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

avian disease, diagnostic, international testing, endemic, virus isolation

Animal types	Life stages
Chicken eggs	embryo
Domestic fowl (<i>Gallus gallus domesticus</i>)	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?

To maintain the diagnostic capability of the National and International Reference Laboratory for Avian Influenza and Newcastle Disease and meet statutory requirements as laid down by WOA/FAO. To give a service of enhanced diagnostic testing and to supply high quality diagnostic reagents for identifying both notifiable and endemic avian viral diseases and some other related viral pathogens such as swine influenza and endemic avian viruses. Where there is a requirement to ensure successful inactivation of a virus, innocuity testing involving the inoculation of eggs with a virus that has been inactivated using a reagent specified in annex 4 is performed.

A retrospective assessment of these aims will be due by 8 January 2030

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?

This work is dictated, to a large extent, by current legislation and meeting worldwide standards (World Organisation for Animal Health), and therefore the need to carry out certain testing regimes is essential. Outputs in previous licences have been commended and the identification of new strains of viruses causing outbreaks of disease. This is particularly beneficial to threat definition to improve health and welfare of avian species and the wider animal and human population.

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

Diagnostic evaluation of samples submitted, information to support the diagnosis or negation of viral infection in GB avian populations and the generation of reagents for diagnostic protocols, ensuring they remain fit-for-purpose. Information will be disseminated by publications and sharing products/reagents that will benefit the wider scientific community as well as national and international reference laboratories.

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

The provision of an effective, timely and reliable laboratory service for the diagnosis of viral diseases of birds has a number of potential benefits. In relation to outbreaks of disease in commercial poultry, the rapid detection of viral pathogens in submitted samples assists government, the private veterinarian and/or poultry company in deciding on appropriate action. This in turn will reduce the impact of the disease on the health of the poultry population under investigation, for notifiable disease this will involve slaughter and eradication to protect the national flock. As well as helping protect the UK food chain, the control of notifiable disease is necessary for international trade in poultry and poultry products. Some diseases such as HPAI (high pathogenicity avian influenza) are zoonotic and can infect humans so is also a benefit of protecting human health in the accurate and timely diagnoses of these diseases. As well as the UK situation, the identification and characterisation of Newcastle disease and avian influenza viruses submitted to the International Reference Laboratory, gives these benefits in many parts of the world. In addition, these results provide information on the global situation regarding these two very important transboundary viruses and enable governments and industry to implement appropriate control measures to limit the spread of the disease, improve the health and welfare of animals and protect public health and food security.

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

We are prolific in publishing outputs within the scientific community and will continue to undertake this approach to the dissemination of data. Where products are generated, either antigen or sera, we will share these reagents widely with collaborators and other reference laboratories. Presentations will be made that include direct outputs from this project license.

Species and numbers of animals expected to be used



Domestic fowl (*Gallus gallus domesticus*): 5,650

No answer provided: 60,000

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 infectious agents being investigated are adapted to avian species, due to the importance of this their use is often mandated in internationally agreed protocols e.g World Organisation for Animal Health (WOAH) and enshrined in quality standards such as ISO17025 which is necessary for undertaking statutory testing.

Live birds- From hatch to either generate diagnostic reagent (e.g. sera, erythrocytes) as a diagnostic reagent or to assess antigens, including vaccine components to assess the pathogenicity of emerging viruses Intravenous Pathogenicity Index (IVPI) and the Intracerebral Pathogenicity Index (ICPI).

Embryonated eggs and embryo tissues - As part of a frontline statutory disease response and surveillance activities diagnostic pipeline whereby the presence or absence of disease is assessed in the embryonic bird as an internationally accepted virus isolation protocol. Currently, this is the most sensitive approach to recovering live virus from diagnostic specimens under test. Additionally, embryonated eggs are used as a reliable method to propagate high fidelity virus stocks in the original host species, thereby preventing alterations in the stock induced by replication in a different host system.

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

Live birds- For antibody generation, birds will be inoculated with an immunogen (e.g. killed or inactivated virus) and a blood sample taken to assess antibody responses to the inoculated material. Where antibodies are detected, birds will be placed under terminal anaesthesia and blood collection undertaken. To obtain erythrocytes, depending on the size/species, blood samples will either be taken by cardiac puncture under deep terminal anaesthesia, or from a superficial vein in accordance with permitted blood volumes. For IVPI and ICPI assessment, birds will be inoculated with live virus as per the standard protocol and humanely euthanised when reaching the humane endpoint determined by a defined clinical score system or at the study end.

Eggs- Eggs will be inoculated into a cavity (e.g. allantoic) or onto a membrane (e.g. chorioallantoic membrane) with material under suspicion or material required to generate a diagnostic reagent.

Inoculated eggs will be monitored according to established protocols and embryos euthanised by a Schedule 1 method (normally decapitation or chilling). Euthanasia will be completed before the embryos reach sentience (48h before hatch).

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

Live birds- Antibody generation- Impacts will be minimal and only inoculation and blood sampling will occur before termination by a schedule 1 method.



Live birds- Assessment of pathogenicity- Impacts may be significant as birds will be infected either by an intravenous route for avian influenza pathogenicity assessment (IVPI- Protocol 4) or intracerebrally for Newcastle disease virus pathogenicity assessment (ICPI- Protocol 5). Clinical disease will likely develop, and birds may succumb to infection. Hence this activity is considered severe. The time period that birds suffer the effects of infection will be minimised by frequent checking and adherence to strict humane clinical endpoints.

Embryos- Virus isolation and propagation for diagnosis - Mild –Most embryos will experience subthreshold severity and it is unclear whether embryos will suffer pain following virus infection as they will be humanely euthanized prior to sentience (48h before hatch). Exceptionally, for some endemic viral diseases, embryos may be kept until just before hatch and then terminated by a schedule 1 method. For this reason, this protocol has a mild severity designation.

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)?

Live birds- Antibody generation- Mild 100%

Live birds- Assessment of pathogenicity (IVPI/ICPI)- Severe up to 50%, mild 50 - 60% based on previous PPL but as these techniques are used for the diagnosis of virus pathogenicity of emergent virus strains during next 5 years this is likely to change.

Embryos- Virus isolation- Mild 20% Subthreshold 80%

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

- Killed

A retrospective assessment of these predicted harms will be due by 8 January 2030

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?

A complete biological system is required for the production of diagnostic material appropriate for the avian host species infected and to meet statutory requirements for diagnosis of infection. Many of the avian viruses investigated are constantly evolving, requiring continual verification that reagents are fit- for-purpose and if this not the case, updating of reagent materials.



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

Throughout the life of the previous licence we have reduced usage of protected animals by developing alternative in-vitro systems (for example cell culture) and have reduced the requirement for eggs significantly.

A review of the market and literature did not identify any availability of replacing polyclonal antisera raised in animals with an animal free alternative. This review will be undertaken regularly during the lifetime of the licence but the organisation is looking at a project to replace antisera raised against a simpler virus using non-animal derived antibodies (either with phage generated multiclonal, using human gene library or mass spectrometry and proteomics approach). However, these approaches are still in their infancy for the replacement of polyclonal serum and success is not guaranteed particularly for complex viruses (such as avian influenza) so as such live birds will be required until a breakthrough is made which with optimistic timescales will only come at the end of this licence period.

Why were they not suitable?

For many tests prescribed by international standards for avian disease control there is still a statutory requirement for the use of animals. For all activities in the licence a complete biological system is required.

The non-animal derived antibody project/technology is still at early days and still has to prove a multiclonal based on human gene library (or other technology) meets the complexity of multiple antigens recognised quality standard by a polyclonal produced in the host species. This is required for the quality standard which is necessary for regulatory testing to avoid issues with specificity (false positives) and sensitivity (false negatives) when used for testing for differentiating different strains of the virus. The speed of this technology in producing antibodies may also rule it out for antibodies required for diagnosis of emerging strains due to short timescales needed for diagnostic response.

Therefore, currently non animal derived antibodies are currently not considered suitable. However, the institute has recently been approached by a company trying to develop this approach and so will engage in the hope of progressing this for these pathogens.

Molecular methods for predicting pathogenicity are being developed and may replace the statutory in- vivo tests used in this licence during the life-time of this licence. Alternatives to the IVPI and ICPI tests can only be fully replaced once the technique has been accepted in to international legislation. A recent publication has evaluated the utility of assessing genetic signatures to define pathogenicity for avian influenza viruses. However, whilst the main driver of pathogenicity is linked to residues at a defined cleavage site, it is widely accepted that further elements that affect outcomes of pathogenicity are present outside of this site and these need to be defined and accepted by the regulatory authorities, therefore IVPIs and ICPIs will still have to be undertaken.

A retrospective assessment of replacement will be due by 8 January 2030

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 animals used on this licence is primarily demand-led by the diagnostic need, with the numbers of animals used for in vivo diagnostic tests being dictated by international standards.

Molecular methodology has been developed to reduce the number of times in vivo tests have to be carried out (e.g. only for the index case of a notifiable disease outbreak to enable initial full virus characterisation). In addition, animals from one of the protocols may be re-used in a second protocol to reduce numbers of birds needed to produce antisera.

Numbers of animals used is predicted based on previous seasons (covered by a previous project licence) where avian notifiable disease outbreaks have driven the need to use animals as part of the diagnostic algorithm.

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

The numbers of animal used on this licence is primarily led by the diagnostic need, with the numbers of animals used for the in-vivo diagnostic tests being dictated by international standards.

World Organisation for Animal Health Online Terrestrial Manual- see section 3.3 (<https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/>)

However, molecular methodologies, such as PCR, have been developed to minimise the number of times the in vivo diagnostic tests have to be undertaken (e.g., only for the index case of an outbreak to enable initial full virus characterisation).

The numbers used in each ICPI and IVPI test are also dictated by the WOAH Manual: <https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/>.

International collaboration with other reference laboratories enables determination of whether IVPI/ICPI are necessary on any novel emergent virus. To reduce the numbers of birds used overall in this licence in IVPI tests, birds that show no more than moderate severity, may be re-used in protocol 3 to raise antisera against the virus.

The protocols on the licence are reviewed, and tests are withdrawn if not in use. The development and provision of tissue culture cells from a wide range of avian and other species improves our diagnostic capability to isolate and identify viral pathogens. This has led to a reduction in the number of embryonated avian eggs required for virus isolation and propagation from 585 a week to 310 during the term of the last licence (a 47% reduction). The number of chickens used to provide erythrocytes for testing has also reduced by 20% from 20 to 16 a week, and further wherever possible surplus reagents are shared with teams able to utilise them. This includes one day old chicks where the number of one day old chicks supplied has reduced by 33% from 13 chicks three times a week to the same



number twice a week. Those not used as cell birds (for erythrocytes) or for IVPI/ICPI are used for serum production, so very little wastage occurs.

The collection of avian blood products is directly matched to use in tests, and the production of antibodies and antigen is done in batches based on demand forecast and preserved to maximise future use.

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

The requirement for the use of animals in diagnostic assessment for notifiable avian diseases depends on submission of samples as a result of clinical suspicion. As both a National and an International reference laboratory for avian viral diseases, we work with other reference laboratories to share approaches and options for reduction in animal and egg use. We use intel from global sources and promote sharing of information to optimise animal and egg use. We also co-ordinate to share reagents and information, thereby avoiding duplication of animal work. Standard protocols are harmonised between diagnostic labs, in order to ensure comparability of results and reduce the need for repeat testing in multiple labs.

A retrospective assessment of reduction will be due by 8 January 2030

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 main animal model used within this licence is the embryonated egg. The embryonic form is used and most of the diagnostic tests are concluded before the embryos reach 2/3rd incubation (sentience).

The production of antibodies and antisera, and the collection of blood products use birds as these are dictated by the diagnostic test protocol.

Chicks and young birds are used for the two statutory in-vivo diagnostic tests (intravenous pathogenicity index (IVPI) and Intracerebral pathogenicity index (ICPI)). These tests must be undertaken in high disease containment facilities where it is impossible for continuous direct physical observation and checking (CCTV allows more regular indirect observation and retrospective monitoring). Birds are monitored physically twice daily for clinical signs with a third evening check added as signs, scored according to a standardized system, reach a moderate level. If the score indicates that a chicken is approaching the severe



endpoint and will not recover from the infection it is euthanised. The proportion of birds that have to be euthanised varies with the pathogenicity of the virus, the lower pathogenic viruses are around 20% but the highly pathogenic viruses it is around 70%. Due to the unpredictable and rapid nature of the disease development following infection with some viruses some birds may die between observations. This information is not known prior to the test being conducted and is a critical component of test outputs for IVPI and ICPI.

A recent publication has evaluated the utility of assessing genetic signatures to define pathogenicity for avian influenza viruses. However, whilst the main driver of pathogenicity is linked to residues at a defined cleavage site, it is widely accepted that further elements that affect outcomes of pathogenicity are present outside of this site and so until an alternative approach is developed it is likely that IVPIs and ICPIs will have to be undertaken.

Molecular methods for predicting pathogenicity are being developed and may replace the statutory in- vivo tests used in this licence during the life-time of this licence. Alternatives to the IVPI and ICPI tests can only be fully replaced once the technique has been accepted in to international legislation.

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

Wherever possible, the main animal model used within this licence will be the embryonated egg and attempts are made to conclude diagnostic tests before the embryos reach 2/3rd incubation.

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

Veterinary assessment and regular monitoring will help identify methods that might reduce welfare costs to animals. The majority of activities will involve embryonated eggs in the incubation phase before sentience. Live bird work will be of a mild severity rating in the main with the exception of the intravenous or intracerebral pathogenicity index tests that are internationally defined, standard diagnostic tests for the quantification of myxovirus virulence according to an internationally agreed scale. Increased monitoring will help refine these activities and clinical disease from IVPI is often rapid. Pain management options are not considered suitable as they could potentially extend the clinical endpoint used which are as per WOAHP methodologies. Under WOAHP methodologies, where clinical endpoints are reached during the day birds are terminated and marked off as a mortality record for the next morning as per the scoring system. Further, methods are standardised to ensure that myxovirus virulence can be compared between viruses isolated in different times or locations, without the need to include control groups for each test.

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

We will use the ARRIVE guidelines in all experimental designs developed and proposed under this project:

<https://arriveguidelines.org/>

Updates to global methods, where available, will be followed in the delivery of in vivo studies. Critical to this is exchange of information between reference laboratories as well as the WOAHP manual and the methods described therein for the diagnosis of avian notifiable disease



https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/A_summry.htm

Further, we will keep up to date with scientific publications in peer reviewed journals for insights into novel methods of diagnostic evaluation that might lead to reduced use of animals and their products.

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

Regular updates from the NC3Rs webpage as well as keeping up to date with advances in the field. Our network of veterinary excellence that supports all animal work at the organisation will also guide where modifications may be developed that apply to this license or where novel techniques such as molecular diagnostic techniques such as next generation sequencing which replace or reduce the need for animal use in this license.

A retrospective assessment of refinement will be due by 8 January 2030

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. Treatment and Prevention of Auto-immune and Immune-mediated 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
- Development, 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

Uveitis, Psoriasis, Systemic Lupus Erythematosus (SLE), Multiple Sclerosis, Thrombocytopenia

Animal types	Life stages
Mice	adult, aged
Rats	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.

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?

This programme of work aims to develop new therapies to treat auto-immune disease and other health conditions associated with the immune response. A new treatment must be both safe and effective. These lead candidates will be assessed for their effectiveness against a placebo (no active compound) control and other drugs currently in use for similar



conditions in the clinic.

A retrospective assessment of these aims will be due by 9 January 2030

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 main factor that is driving the need for improved autoimmune therapeutics is the large number of people affected. There are more than 80 autoimmune disorders affecting 5 to 8% of the population worldwide. This signals the need for novel therapies in this area and a better understanding of the mechanisms behind immune-mediated conditions.

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

By providing high quality services in this important area, supported by scientific expertise, we can make the testing of such drugs more cost effective, more informative and reduce the need for companies to set up these models in house, a process requiring many animals to be used.

The data generated under this project will provide information on lead compounds' efficacy:

New information on beneficial effects when compared to a vehicle

New information on beneficial effects when compared to a reference drug

All data generated will be analysed prior to being recorded in our archives (electronic files and/or hard copies). All data from a given study will be compiled into a report comprising: (i) a short introduction including the aim(s) of the study, (ii) a materials and methods section including an outline of the experimental protocol, a summary of the experimental groups and a description of each readout, (iii) a results section including graphical representations of the analysed data, statistical analyses and interpretations, (iv) a conclusion section with a summary of all findings and (v) a raw data section. The study outcome, whether judged 'positive' (e.g., beneficial effect of the lead compound when compared to a reference drug) or 'negative' (e.g., no beneficial effect of the lead compound when compared to a vehicle and/or no greater beneficial effect of the lead compound when compared to a reference drug and/or observed adverse events) will be reported.

The data generated will typically be sufficient for the study sponsor to decide the lead compound's future: (i) pre-clinical toxicology, the next step in the drug discovery process leading to a novel therapy for an autoimmune or inflammatory disease, (ii) additional efficacy studies or (iii) rejection for lack of safety or efficacy.

Our expertise allows the study sponsors to obtain advice on the most suitable model to test their lead compound. The interactions between us and the study sponsors at the study



design stage and during the studies will allow preventing, anticipating or rapidly reacting to potential adverse events.

Some data may be used to generate scientific literature. These data are made available to the scientific community (i) during scientific conferences attended by us or our clients, (ii) upon request through our website or (iii) through targeted email campaigns and social media.

Some data may be used for meta-analysis in order to refine experimental designs and experimental methods and to reduce the number of animals used.

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

By contributing to the development of new candidate drugs for the treatment of autoimmune diseases and inflammatory diseases, our project will benefit patients improving their quality of life.

Some data may be used to generate scientific literature to benefit the scientific community. These data are made available to the scientific community (i) during scientific conferences attended by us or our clients, (ii) upon request through our website or (iii) through targeted email campaigns and social media.

Some data may be used for meta-analysis in order to refine experimental designs and experimental methods and to reduce the number of animals used.

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

The outputs of this work will help guide next steps. Where possible data will be shared at conferences, social media and in publications. The data will aid future studies, add to our experience to advise clients and provide information to other scientists.

Species and numbers of animals expected to be used

- Mice: 3000
- Rats: 500

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.

Many different strains of both rats and mice have been established which provide models of a range of human diseases. Due to their size, ease of reproduction and handling, many aspects of the immune response, leading to disease caused by inflammation and autoimmunity, have been well characterized. The genetics of these strains is well understood, aiding our understanding of disease development and potential interventions. Thus, mice and rats, are, for many diseases, the most appropriate species for early assessment of potential novel therapies.

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



Animals used in this project typically either develop aspects of the disease of interest spontaneously or following disease induction by administration of a disease inducing chemical or antigen associated with autoimmunity.

Alternatively, wounds may be created for assessment of improvements in the wound healing process. This typically involves surgery to create partial or full thickness wounds. Surgery will be carried out under anaesthesia and will be monitored throughout and in recovery. Pain killers will be administered as required, either by injection, on the skin or in palatable gel.

Treatments will be administered either prophylactically or therapeutically by routes which in themselves will cause only minor transient discomfort and will involve anaesthesia, with recovery, as required.

Animals will be monitored throughout for development of disease. Bodyweights will also be measured.

Blood samples will be taken, in-life, for assessment of immune response, involving restraint for a short time; rats will be acclimatized to restrainers in advance to minimise the potential stress involved.

Where test articles are suitably labelled with eg., fluorescent tag, animals may be imaged, under anaesthesia, for detection of the fluorescence to show the distribution of compound following administration. Immune cells may also be assessed by imaging following uptake of fluorescent dye.

Animals will be killed at the end of the study by a humane method, or while under end-of-life anaesthesia to allow for flushing animals with e.g., saline or preservative or collection of blood.

Duration of studies will typically be 4 to 6 weeks but varies greatly depending on the model, especially where spontaneous models are used but typically last 4 to 6 weeks, e.g., Lupus, technically known as systemic lupus erythematosus (SLE), which is an autoimmune disease in which the body's immune system mistakenly attacks healthy tissue, causing, eg., painful and swollen joints, fever, chest pain, hair loss, mouth ulcers, swollen lymph nodes, feeling tired, and a red rash which is most commonly on the face. Models of SLE may last up to 40 weeks, while models of eg., thrombocytopenia, a blood disorder which results in low numbers of the blood clotting cells, platelets, may last 6 days, with peak disease at 48 hours.

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

Generally, the compounds used for disease induction cause no more than transient discomfort, however, some substances such as Imiquimod, for induction of Psoriasis, do cause irritation and some discomfort. Similarly, formation of wounds for analysis of wound healing processes will also cause some discomfort. Such situations will be monitored and where required analgesics may be administered directly or in food (gel).

Autoimmune diseases may be induced by administration of proteins, associated with disease induction, administered together with an immune stimulatory compound.



This combination may, itself, cause some localized swelling, which will need to be monitored, and the animal killed if the swelling is deemed to cause pain, discomfort or effect normal activity.

In some cases, the chemical may cause some mild, transient adverse effects including bodyweight loss. Additional nutrients may be supplied in the form of gel or mashed food on the cage floor as appropriate.

Animals developing autoimmune or immune-mediated conditions will vary greatly in their experience from no clinical signs to moderate levels of clinical disease.

Induction of an autoimmune retinal disease in mice (experimental autoimmune uveitis, EAU) results in retinal inflammation which can be observed by microscopy.

While disease may affect vision, the animal otherwise continues to behave normally, remaining active, grooming, interacting with cage mates and continuing to grow as expected for their age.

In contrast, those animals receiving antigens associated with induction of multiple sclerosis-like disease in mice, a model of experimental autoimmune encephalomyelitis (EAE), are expected to show weight loss prior to onset of disease which leads to paralysis, initially the tail, then hind limbs and in severe cases the forelimbs. Animals show mild clinical signs of EAE including bodyweight loss, tail limpness and loss of movement of the limbs for three to five days, from around 5 days following administration of the disease inducing agent. Disease severity then increases to peak after three to five days. At peak disease, animals show hind limb paralysis - both hind limbs can be affected. Often, peak disease lasts three days.

The severity of clinical signs then decreases rapidly, and animals return to normal, with only mild clinical signs or no clinical signs at all. The appearance of clinical signs is always progressive: tail limpness always precedes hind limb paraparesis or paralysis. Front limbs are not typically affected. Typical study duration is four weeks.

Where chronic, recurrent disease is involved animals will experience an additional period of paralysis lasting 2-3 days.

Spontaneous models of SLE will be terminated prior to showing adverse effect due to monitoring levels of the immune response to proteins that are naturally expressed in the body and high levels of protein in the urine, an indicator of kidney damage, which may occur from 4 months of age.

Some models of SLE may be associated with other indications e.g., the spontaneous MRL/lpr mouse model and the pristane-induced model may develop arthritis from 2-3 months of age. Additional checks for signs of arthritis will be included in these models. In addition, abnormalities on the skin, as well as abdominal distention may be observed and will be monitored.

Induction of Psoriasis leads to reddening of the skin, skin flaking, erythema and skin thickening which develops over the course of disease induction. Generally, animals experience mild to moderate clinical signs due to skin irritation.

Thrombocytopenia, a condition in the blood, in which the population of cells required for



blood clotting (platelets) is reduced, can be chemically induced, leading to a reduction of platelet counts over several days. Induction of disease may cause an increased risk of bleeds and an initial adverse reaction.

Animals will be monitored closely for the first few hours and the extent of thrombocytopenia will be regulated to minimise risk of spontaneous bleeds.

Wound models may show initial clinical signs in response to surgery and signs of discomfort due to wound, but adverse effects should not last more than 24 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 animal type)?

For the majority of the models described, animals will be terminated before developing signs of clinical disease, with approximately 40% experiencing mild adverse effects, and 60% moderate severity.

Induction of EAE does lead to >80% of animals experiencing severe disease due to developing paralysis, although transient, lasting 2-3 days.

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

- Killed

A retrospective assessment of these predicted harms will be due by 9 January 2030

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?

Immunology looks at how the immune system responds to stimuli and involves multiple systems, multiple organs and multiple cell types. The complexity of the immune response cannot be reproduced in laboratory tests alone as there is often interaction between the different components, which are not always obvious.

Experiments on cell lines and on cell cultures will be performed to help understand the potential of a compound to interact with immune cells. 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 and progress potential therapeutics to the clinic.



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

Clients will often provide data from assays already performed that indicate the potential of a compound to reduce the onset of immune-related disease. In the absence of such data, in vitro assessment may be required before progressing to an animal model. We have a number of in vitro cell based assays including use of transwells and 2D models. We are also developing the use of organoids and tissue on a chip models for assessment of test compounds prior to testing in animals.

Why were they not suitable?

Alternatives to animal models are a great addition in the assessment of test compounds and can help to confirm mode of action. Most test compounds have been assessed in vitro by the sponsor or by our Immunology Team, however due to the additional complexity of the human body and interactions of immune cells and pathways, it is not possible to replicate this in vitro, and thus in vivo studies are unfortunately still required to fully understand and show efficacy.

A retrospective assessment of replacement will be due by 9 January 2030

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 is estimated from previous animal returns.

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

Research staff are trained in Experimental Design and Statistical Analysis of Biomedical Experiments. Power analysis will be performed using G*Power (Faul F. and Erdfelder E., 1992) to establish the total sample size required to generate meaningful data. The data we have accumulated since 2004 allows post hoc power analyses to be performed but a priori analyses are still used when validating new efficacy or potency models. The Standard Operating Procedures (SOPs) in place make it possible to perform meta-analyses of data from strictly comparable studies and generate more accurate effect size values to feed the power analysis software.

Typically, power value will be set at 80% to reduce the number of animals used in the studies while still maintaining a high likelihood of detecting any effects of compound.



An experimental protocol is drafted prior to each study. A typical experiment to validate a new model will include one or two groups of animals in which disease will be induced or arises spontaneously and a group of non-diseased animals. A typical experiment using a validated model will include five experimental groups: a group of vehicle-treated animals, a group of animals treated with a reference drug and three groups of animals treated with ascending doses of a lead compound. Naïve animals will only be included for comparisons when tissue samples and/or blood samples are required for ex vivo assays. The number of naïve animals is typically limited to three animals. The models listed in the licence have been validated but may require further validation if they haven't been run for >12 months or a different strain of mouse is to be used. Sample size (total number of animals in a study) and thus number of animals per group are calculated using the power calculation software G*Power.

While we work to GLP-like standards, however, we do not have to comply to additional regulatory guidelines.

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

Understanding from the literature and breeding establishments the expected incidence of disease and variability of the model will help ensure group numbers are appropriate. Assessment of previous data or review of the literature will also help guide the group sizes as well as ensuring appropriate control groups included. Where suitable, previous experimental data from our establishment will be used to allow for comparison. In addition, if possible, we encourage the use of a shared control group among different studies using the same model.

A retrospective assessment of reduction will be due by 9 January 2030

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.

Animal models that develop clinical signs of disease similar to that of human diseases are required for assessment of potential new improved therapies. While potential new compounds can be tested in other systems before being used in animals, the complexities of the immune system make assessment in animal models necessary. Animal models described in this license have been developed in a number of different species including mice, rats, rabbits, cats, dogs, pigs, sheep, horses and primates. Only rodents (mice and



rats) will be used under the Protocols listed on this license. For some disease models particular strains will be used that develop clinical disease spontaneously or are particularly susceptible to disease inducing agent. Further some models may use genetically modified animals that are more prone to disease. The protocols described below have been established over many years and cited in peer-reviewed journals as reliable and well defined for the disease model of choice, these are therefore more reproducible, reducing the number of animals required and well defined for the disease model of choice. Mice are the species that will be used by default. Rats will only be used when the inflammatory disease cannot be modelled in mice, when the test compound is not well distributed in mice or when the blood volumes or tissue quantity required for multiple assays are not compatible with sampling from mice.

EAE, SLE, EAU, Psoriasis, Thrombocytopenia and wound healing are complex auto-immune diseases or involve complex immune interactions. While some individual aspects of these processes can be modelled in vitro, the complexity of the interactions between multiple body systems that is critical to understanding these conditions and in defining treatments cannot.

Efficacy models listed in this license are validated models. The methods have been published in peer-reviewed journals by academic or industrial groups. The models have been used to test the efficacy of drugs currently on the market.

Inflammation of the retina in EAU can be further studied by topical endoscopic fundus imaging (TEFI). Animals do normally need to be restrained during imaging acquisition.

However, this can be cause of stress and therefore, they may be anaesthetised for imaging acquisition.

The mildest disease inducing agent or dose will be chosen during the planning phase limiting the study duration as much as possible without compromising the aim of the study.

Where minor surgical procedures are performed, for example, insertion of minipumps, analgesics will be given in accordance with veterinary advice.

Where severe models are required to assess potential therapeutics to reduce specific disease symptoms in patients, animals will be monitored closely and be provided with additional enrichment or care as required.

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

Most of our studies will be done in mice, which are the lowest species that develop these diseases in the same way as humans. Rats are occasionally used when the disease cannot be modelled in mice, if the test compound does not work appropriately in mice, or if a larger animal is needed for sampling requirements.

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

Animal suffering will be limited by ensuring that the models used cause the least amount of harm to the animals. The mildest disease inducing agent or 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.

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

We follow the recommended Best Practice guidelines of Turner et al. 2011 and Diehl 2001. We keep up to date by receiving regular updates from the Home Office (HO), LASA, as well as the IAT, following the literature, internal forums and advice from our dedicated veterinary surgeon.

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

We keep up to date with NC3Rs guidelines by online updates as well as attending Institute of Animal Technology (IAT) and Laboratory Animal Science Association (LASA) conferences, Home Office Liaison, Training and Information Forum (HOLTIF) and workshops.

We also check the literature to ensure the models we are using are the most appropriate and include additional validation of models where improvement can be made.

A retrospective assessment of refinement will be due by 9 January 2030

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. Diagnostic and cerebroprotective strategies in preclinical models of 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

stroke, cerebral ischemia, neuroprotection, intracerebral haemorrhage, biomarkers

Animal types	Life stages
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 project aims to identify novel biomarkers and new treatments for stroke, a common condition that damages the brain and causes high levels of mortality and disability.

A retrospective assessment of these aims will be due by 18 January 2030

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?

Currently there are only two treatments for ischemic stroke. Both rely upon removing the blockage from the affected blood vessel in the brain. However, these treatments are not widely available and are time- limited (3-4 hrs after stroke onset) as salvageable brain tissue is often lost by this time. The other type of stroke – haemorrhagic stroke – is less common (15-20% of strokes) but suffers from higher mortality and morbidity and has limited treatment options. We therefore need new diagnostics to better identify people with strokes so that they may be directed to specialist stroke units, and treatments that can prolong the survival of brain tissue to make existing treatments viable for more people and improve outcome after stroke.

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

Outputs will take the form of:

- Novel empirical research data that we will generate during the study
- Scientific papers that we will publish Open Access in reputable peer-reviewed journals
- Information that we will share with the general public in readily accessible forms
- Knowledge as to the potential of blood purines as diagnostics for strokes
- Knowledge as to any benefits of our therapeutic approach in stroke.

These outputs will be communicated to relevant stakeholders in the following ways:

- We will present our findings at invited seminars and scientific conferences.
- We will publish our findings in open-access peer-reviewed journals.
- We will communicate our findings in non-specialist terms to those affected by stroke through both written materials (eg web-based) and in-person meetings (eg with the stroke expert-by-experience community).
- We will liaise with biotech companies to pursue research and clinical development of any effective biomarkers so that scientific advances can be translated promptly.



- We will liaise with clinicians so that potential treatments we identify can be pursued through clinical trials so that patients may benefit.

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

The scientific community will benefit from the new knowledge we generate.

The general public will benefit from opportunities we will offer to talk about stroke, its diagnosis and treatments based on our studies in this PPL.

Pharmaceutical and biotech companies may benefit from developing the therapeutic and diagnostic strategies we propose to study.

Clinician may benefit through having new diagnostic and therapeutic tools for stroke.

Ultimately those suffering from stroke may benefit from more rapid diagnosis and better recovery from their stroke.

The short-term benefit will be the scientific data we generate and disseminate, and the public awareness we raise of stroke, its causes and treatments.

The long-term benefit of our goal is to arrive at a new biomarkers and treatments for stroke that would benefit the many people in the UK and across the world that suffer from a stroke.

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

We will collaborate with relevant experimental stroke research groups in the UK and abroad to widen the range of models in which the treatment may be effective and to ensure reproducibility.

We will publish our findings - including our negative results - so they can be scrutinised, successful results replicated and unsuccessful approaches avoided by others.

We will engage with stroke patient/carer groups and stroke charities to share the findings that emerge from this project, and more generally with the public to increase public awareness of stroke.

We will engage with pharmaceutical and biotech companies to i) develop minimally invasive (eg finger prick) diagnostic tests for stroke and ii) arrive at formulations that can be used in clinical trials.

We will protect any IP that emerged through patent and licensing.

Species and numbers of animals expected to be used

- 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.

Adult rats are very commonly used in experimental stroke research; the models are very firmly established and have good validity for human stroke. Rats are preferred to mice given the wealth of rat stroke literature that we can turn to for guidance and to avoid unnecessary replication, their larger size facilitates many of the experimental manipulations proposed in this project, and their greater intelligence compared to mice facilitates behavioural testing through reduced variability. In addition, due to naturally-occurring mutations, some strains of rats spontaneously display the comorbidities commonly associated with stroke in humans, such as high blood pressure, making them a better model for stroke. There are also many rat and rodent stroke groups with whom we could share our experiences and best practice and collaborate with them to further the aims of this project.

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

A rat will be given a stroke, an event that occurs naturally even in pet rats. To give a rat a stroke it is common to block a blood vessel in the brain with a nylon filament. This stops the blood flowing past the blockage, exactly as it happens in humans suffering a stroke. Indeed, the blood vessel that is commonly blocked in rats is the same one that is often blocked in humans (the middle cerebral artery). The blockage is often confirmed through the use of a cerebral blood flow-monitoring device, which occasionally may require a craniotomy to be performed. The blockage can be permanent, but in our study will be temporary (typically an hour or less), mimicking the successful return of blood flow to the brain after treatment. This reduces the severity of the stroke on the rat. Alternatively, a compound causing a bleed in the brain (haemorrhagic stroke) will be delivered into the brain itself via a craniotomy. A rat will only receive one stroke and will be carefully monitored after its recovery from the anaesthetic under which the stroke was delivered. To establish the benefits of our cerebroprotective approach, compounds may be delivered through an indwelling intravenous cannula or by other means (eg subcutaneously). To measure potential blood biomarkers associated with ischemic and haemorrhagic stroke, peripheral (eg tail vein) blood samples may be taken at regular intervals before, during and after the stroke. Post-stroke, the rat will exhibit many of the clinical signs that humans do after a stroke and which we can assess with a variety of neurological and behavioural tests, which are not harmful to the animal. Some tests for neurological function (eg grip strength, limb placing) are performed ~24 hrs after the stroke, while others are conducted in the days and weeks after the stroke. These tests, conducted over a period of 28 days after the stroke and typically up to 4 tests per day, and the appearance of the brain will tell us if our treatment is successful in reducing the damage caused by stroke. At the end of



the study the rats will be killed and their tissues used for detailed examination, maximising the scientific benefits from each animal.

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

All surgical procedures will be conducted under deep general anaesthesia. Thereafter, while the effects of stroke are not painful, there will be pain associated with the surgery which will be treated by intra- and post-operative painkillers for as long as is necessary, typically two days. Rats with a stroke will likely eat less and lose weight (up to 20% of pre-stroke body weight) over the 4-5 days after a stroke, so are encouraged to eat with attractive food such as peanut butter, apple sauce and trail mix that they will be habituated to ahead of time, and placed conveniently for them on the floor of the cage. There will be abnormalities of behaviour - especially movement as one side of the body will be affected, as it is in humans. However, this should not normally prevent the animal from moving, grooming, eating or drinking, especially two or so days after the stroke. While rats learn to compensate for these impairments, since brain repair is limited, there will be permanent impairments that can be picked up with behavioural tests - we hope that our treatment reduces the extent of these impairments.

Occasionally (~10%) strokes have severe impacts on the brain and result in adverse effects such as tonic-clonic or barrel rolling seizures, which would represent humane endpoints for the animal, as would weight loss >20%.

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)?

Given the expected post stroke weight loss and neurological impairment, we expect the majority (~80%) of animals receiving a stroke to be in the Severe category. There will be instances (~20%) where the stroke has not affected a large part of the brain for technical or cerebrovascular anatomical reasons and the impact of the stroke will be less (Moderate). Occasionally the stroke will result in a large amount of brain damage (as it does in humans). The consequences expected include severe weight loss (>20% of body weight) and tonic-clonic or barrel-rolling seizures. We would expect to see this in about 10% of animals and these animals will be humanely killed as they had reached a humane endpoint, but tissue would be analysed so that we may obtain some useful information from these animals. A spontaneous mortality rate of ~10% is expected, and again tissue would be analysed, including to ascertain the cause of death. The recovery stroke studies described above will occur in the majority (~60%) of animals used in this PPL (Protocols 3 and 4). The remaining animals will be used for studies such as behavioural or drug testing, blood measurements and non-recovery strokes where the severity in the majority of cases will be Mild.



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

- Killed

A retrospective assessment of these predicted harms will be due by 18 January 2030

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 there are test-tube and computer models of the brain, we cannot use these models to replicate what happens in an intact animal when it suffers a stroke, nor can we use these models to predict the response of these animals to novel treatments, especially in terms of what biomarkers may be released by the stroke brain. This is because every organ system is involved, from the nervous system, the cardiovascular system, the respiratory system and the liver in dealing with novel compounds.

Predicting the extent of brain injury and the impact on cognition and behaviour are impossible without the use of a live animal, and nor could we guess if our treatments may be effective. Thus, while we used test-tube models in the past, the time has now come to test our hypotheses in the whole animal.

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

We extensively used test-tube models of brain injury (developmentally mature acute brain slices and neuronal cell culture) and computer models of the relevant biochemical pathway to arrive at this point. Brain organoids and brain on a chip approaches were not available at the time of the early proof-of- concept studies, but may be of value in future in detailed cellular mechanistic studies, particularly in neonatal stroke (as opposed to adult or geriatric stroke) that such tissues are more likely to more closely resemble

Why were they not suitable?

There is a limit to how much information test-tube and computer models can give, and they cannot model or predict the release of potential diagnostic biomarkers into the blood stream from the brain, nor the functional sensorimotor or cognitive outcomes for novel compounds in a complex and dynamic mammal that is responding to a brain injury.

A retrospective assessment of replacement will be due by 18 January 2030



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 four Protocols, each of which assess different aspects of the project. Based on previous experience and that in the published literature, some aspects, due to the variability in the extent of the stroke and the outcome for the animal, will require larger group sizes, especially when animals have to be removed from the study due to experiencing a severe stroke. This, coupled with the number of control and treatment groups, leads to large numbers of animals, typically 12 – 16 per group.

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

We based our calculations on the previous pilot study we conducted. This gives us a good estimate of the variability we see in our experiments and allows us to predict the number of animals that are needed to achieve robust, statistically-significant findings of that type that would be credible to fellow scientists, funding agencies and companies that we wish to engage with. As such, using too few animals is just as bad as using too many as the former would not provide the assurances as to the veracity of our observations, and thus those animals would have been used without benefit.

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 buy in wild-type and co-morbid rats (eg those prone to hypertension) from reputable and recommended suppliers on demand instead of having in-house breeding colony on site to avoid wastage from risk of overbreeding/underuse and also to prevent genetic drift.

We will also use tissue from animals for a number of studies, and share with colleagues.

A retrospective assessment of reduction will be due by 18 January 2030

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 shall primarily use a rat model of transient occlusion of brain blood vessels - most commonly the middle cerebral artery. This model has been highly refined over the years and much is known as to the surgery necessary, the appropriate occlusion methods and the duration for optimal survival and reduced incidence of adverse outcomes. Confirmation of the reduction in blood flow can use techniques avoiding craniotomy such as placement of the probe on the thinner temporal bones of the skull. Alternative models for cerebral artery occlusion involve the injection of vasoconstricting substances into the brain in the vicinity of blood vessels and to induce pharmacological, as opposed to mechanical, occlusion of the blood vessel. This is less clinically-relevant than the filament model, but avoids the neck and vascular surgery associated with filament occlusion, and facilitates simultaneous optical, electrophysiological and biosensor recordings of the effects of a stroke on the brain before, during and after the stroke in non-recovery mechanistic studies. Such longitudinal measures in which the same animal acts as its own control increases the statistical strength of the observations.

Haemorrhagic stroke is commonly induced by the introduction of blood or a bleed-inducing substance into the brain, such as collagenase, has good relevance to human intracerebral haemorrhage (ICH), and is highly reproducible based on the known volumes of blood or collagenase injected, reducing variability and the risk of adverse outcomes, and potentially the size of experimental groups.

Standard blood sampling (eg tail vein) will typically be used to measure blood biomarkers, but ongoing developments in sensor technology may require alternative routes and approaches (eg indwelling or skin-applied sensors, of the type used in blood glucose-sensing in people with diabetes). Potential cerebroprotective compounds will be delivered by routes and durations of translational relevance to humans, primarily intravenously, and over extended periods of time (up to 8 hrs) or via sub/cutaneous patches, which may be left in place for a period of days. Indwelling (eg femoral) cannulas provide a convenient route that can be diverted away from access by the animal (eg via subcutaneous tunnelling to the dorsal neck region) and can be sealed, with, for example an appropriate



cap to minimise risk of infection, and left in place after infusion to avoid a second surgery. Handling of the animals prior to the surgery will facilitate the post-surgical sealing of the cannula.

When possible, animals will be habituated to behavioural tests or environments to minimise any stress associated with the test. Animals deemed not capable of performing a test will not be put through test and scored zero for performance.

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

We do plan to use terminal anaesthesia in some studies designed to examine mechanisms, but less sentient stages or species are not suitable for our studies where we wish to study the types of strokes that affect older humans. In this case we need a developmentally-mature mammalian model, including ones displaying the types of co-morbidities seen in humans at risk of stroke (eg high blood pressure), in which we can readily perform the experimental manipulations, the variety of recording modalities and the behavioral tests of neurological and cognitive function. For these purposes, the adult rat, and the co-morbid strains available, are superior to the smaller, less intelligent but less sentient mouse.

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

Through performing surgical procedures and talking with animal facility staff and other colleagues (locally and elsewhere) we can identify potential refinements eg: avoiding two surgical incisions when one will suffice to introduce a cannula; conducting surgical procedures in an order that minimises the duration of surgery; reducing occlusion time; limiting the exposure of animals to behavioural tests; habituating animals to postsurgical food; placing food on the floor of the cage; video monitoring over the first 24 hrs; defined volumes for ICH induction; pharmacological vasoconstrictor occlusion as opposed to mechanical surgical occlusion thereby avoiding extensive neck surgery; avoiding craniotomy where possible, and the use of caps or pins to seal cannulas.

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

There are a number of guidelines that specifically support experimental stroke and more broadly animal research. These include the LASA on general animal welfare, aseptic surgery, administration of substances; The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) for blood sampling, welfare assessment; PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) Guidelines for the conduct of animal experiments; Stroke Recovery and Rehabilitation Roundtable (SRRR) on post stroke behavioural tests, and Stroke Therapy Academic Industry Roundtable (STAIR) and Ischaemia Models: Procedural Refinements Of in Vivo Experiments (IMPROVE) for the conduct of stroke studies. In order to publish in reputable journals, Editors and Reviewers will expect these



guidelines, as well as Animal Research: Reporting of In Vivo Experiments (ARRIVE), to be followed.

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

We receive regular updates from the NC3Rs via the AWERB and our animal facility. We are also in constant contact with our named officers and animal care technicians who alert us to developments in animal welfare that we can readily implement (eg handling by avoiding tail suspension).

A retrospective assessment of refinement will be due by 18 January 2030

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. Development of veterinary medicines for equines

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
 - 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)

Key words

Veterinary medicine, Horses, Equine, Efficacy, Safety

Animal types	Life stages
Horses	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.

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 the programme of work is to develop safe and effective means of preventing and controlling disease in horses and ponies.

A retrospective assessment of these aims will be due by 22 January 2030

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?

To provide data to ensure registration of products in use in equines for prevention and control of diseases in horses and to promote health and welfare of horses.

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

The primary benefit of the work conducted under this licence is the production of reports which are fit for the purpose of submission to the relevant regulatory authority e.g. for a marketing authorisation to enable new substances to be available for prevention and control of disease in equines.

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

The programme of work would contribute to the development of a number of safe and effective products for the prevention and control of disease in equines. As there is a mandatory requirement to conduct studies to demonstrate the safety and efficacy of such products before they can be marketed in the EU and worldwide, the consequences of this work not being carried out would be that safer, more effective products would not be available to treat disease. Whilst it is accepted that other establishments may be able to provide this service, the combination of skilled staff and the large-scale, purpose-built animal facilities make for an ideal site to conduct this type of work.

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

Data and reports from studies are included in dossiers for submission to regulatory authorities to allow for the use of safe and efficacious products.

Species and numbers of animals expected to be used.

- Horses: 250

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 European Parliament and Council Directives 2001/82/EC as amended by 2004/28/EC lay out the Community Codes relating to veterinary medicinal products.

Article 1 of the directive defines a veterinary medicinal product as any substance



presented for treating or preventing disease. Further, any substance or combination of substances which may be administered with a view to making a medical diagnosis or to restoring, correcting, or modifying physiological functions is also considered a medicinal product. The scope of this licence is inclusive of chemical pharmaceuticals, pharmaceutical products derived from biotechnology including gene therapies and monoclonal antibodies, vaccines, serums, allergens, excipients of medicines and foods or feed additives intended for curative purpose or to maintain health.

A new veterinary medicinal product can be placed on the market within the European Union only when the applicant has obtained a national or European marketing authorisation. The Community Codes relating to medicinal products (2001/82/EC, Article 12 as amended by 2004/28/EC) list the particulars and documents that must be provided in support of an application for marketing authorisation. These include the results of physico-chemical, biological or microbiological tests, toxicological and pharmacological studies, and clinical trials. The obligatory components of applications for marketing authorisation listed in Council Directive 75/318/EEC have been refined many times over the last thirty years to keep pace with technological progress and the advance of scientific understanding. Agencies of central government issue test guidelines prepared by national or international experts to ensure a consistent approach and a framework of study designs most likely to achieve the objective of characterising the effects of a proposed new medicinal product. Examples include the guidance notes issued by the European Medicines Agency (EMA) and the monographs of the European Pharmacopoeia.

Other countries and supra-national organisations have also developed their own guidance documents to ensure high standards of assessment of new veterinary medicinal products before they can be distributed to the public. These independently developed and disparate national guidelines have been progressively standardised over a period of decades by experts of many countries working through organisations such as the Organisation for Economic Co-operation and Development (OECD) and the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). VICH guidelines present both detailed specifications of study designs and strategies for combinations of studies that will characterise the effects of a new medicinal product in the most efficient and effective manner. The efforts of VICH have, in part, been intended to reduce the occurrences of demand for a study meeting the requirements of one country to be repeated with the variations of design demanded by another country. The consequent economic savings have been associated with savings in the numbers of animals required for testing purposes. The manufacturers of new medicinal products can now set out to produce a comprehensive dossier of studies conforming to VICH specifications in the expectation that the dossier will address the needs of regulatory authorities world-wide.

Guidance on the conduct of efficacy studies for vaccines and immunosera is available in a range of European Pharmacopoeia monographs and varies considerably between products. The dose used is that quantity of the product to be recommended for use and containing the minimum titre or potency expected at the end of the period of validity. For live vaccines, the most attenuated passage level that will be present in a batch of vaccine should be used. The efficacy evidence must support all the claims being made, for example onset and duration of immunity. For vaccines, the influence of passively acquired and maternally derived antibodies on the efficacy of a vaccine should be evaluated. The efficacy of each of the components of multivalent and combined vaccines should be demonstrated using the combined vaccine. Demonstration of efficacy is usually undertaken under well controlled laboratory conditions by challenge of the target animal.



The challenge should usually mimic the natural conditions of infection, with regard to the amount of challenge organism and route of administration for example. Unless otherwise justified challenge should be carried out using a strain different from the one used in the production of the vaccine. In general, results from laboratory tests are supplemented with data from field trials, usually carried out with untreated control animals.

Data from target animal safety studies are required for registration of veterinary medicinal products in the regions participating in VICH. International harmonisation of standards for essential target animal safety studies facilitates adequacy of data and minimises the need to perform separate studies for regulatory authorities of different countries.

VICH Topic GL44 (Guideline on Target Animal Safety for veterinary live and inactivated vaccines) advises that for vaccines shown to retain residual pathogenicity, overdose testing using a 10X dose is recommended. For each target species, seronegative animals of the most sensitive class, age and sex of animal should be used. If multiple routes and methods of administration are specified for the product concerned, administration by all routes is recommended. If one route of administration has been shown to cause the most severe effects, this single route may be selected as the only one for use in the study. For vaccines that require a single lifetime dose or primary vaccination series only, the primary vaccination regimen should be used. For vaccines that require a single dose or primary vaccination series followed by booster vaccination, the primary vaccination regimen plus an additional dose should be used. For convenience, the recommended intervals between administrations may be shortened to an interval of at least 14 days. General clinical observations for the type of vaccine and animal species should be made every day for 14 days after each administration. In addition, other relevant criteria such as rectal temperature and injection site reactions should be measured. The effect on the reproductive performance should be evaluated if there is likely to be a risk. Vaccines recommended for use in pregnant animals must be tested in each of the specific periods of gestation recommended for use on the label. The observation period must be extended to parturition, to examine harmful effects during gestation or on progeny. Field studies should be conducted under animal husbandry conditions representative of those regions in which authorisation is sought. Two or more different geographical sites are recommended.

VICH GL43 (Target Animal Safety for veterinary medicinal products) advises that margin of safety and other appropriate studies be carried out for veterinary medicinal products. The general design uses multiples of the proposed use dose and duration of administration of the product. Specific dose, frequency and duration combinations should be selected and justified based on the pharmacology and toxicology of the product. Unless otherwise justified the design of the margin of safety study should include negative control, the highest recommended dose level (1X) and two multiples of this (usually 3X and 5X) for a period of time in excess of the recommended maximum duration of use. It is recommended that each group be treated for at least three times the proposed duration up to a maximum of 90 days. Administration for longer than three months may be justifiable, where drug accumulation may increase over time or where duration of drug activity exceeds two months for example. The variables measured are likely to include physical examinations and observations, clinical pathology tests, necropsy and histopathology examinations. Additional safety studies (for example injection site, administration site for topical applications, reproductive safety and mammary gland safety) may be appropriate for a particular product depending on the conditions of use and characteristics of the product. Such studies may be combined with the margin of safety evaluation and, in food producing animals, residue studies.



Bioequivalence studies are often part of applications for generic veterinary medicinal products to allow bridging of safety and efficacy data associated with a reference veterinary medicinal product. The EMA guideline (EMA/CVMP/016/00) provides guidance on the design, conduct and evaluation of bioequivalence studies for pharmaceutical forms with systemic action.

Dose determination and dose confirmation studies are required for demonstration of efficacy of NSAIDs and antimicrobials. Dose determination studies should be conducted in the target species using a range of doses selected on the basis of preliminary studies, parameters that are relevant for the anticipated effect and a dose range that is considered appropriate for further use. Usually a minimum of three doses are included, the central one being the expected recommended dose.

The aim of dose confirmation studies is to confirm the efficacy of the selected dosage regimen under controlled clinical conditions. Efficacy criteria used to assess the outcome of disease / infection should be similar for dose determination and dose confirmation studies. When progressing from dose confirmation to dose determination studies it is possible to add efficacy criteria but not to exclude any.

As part of the process of developing new vaccines and immunosera, antibody production may need to be stimulated in live animals and the antibodies collected. Usually animals will need to be immunised in order to stimulate antibody production and the antibodies may then be collected by, for example, milking or blood sampling.

The efficacy and safety of veterinary medicinal products shall in the first instance normally be demonstrated by experiments under laboratory conditions. However, Council Directive 81/852/EEC and the European Pharmacopoeia state that, unless justified, the results from laboratory trials shall be supplemented with data from field trials. It is also states that, when efficacy cannot be demonstrated by laboratory trials, field efficacy trials alone may be acceptable.

Field trials should cover the major uses. The veterinary medicine should be administered by the most critical route, method and regimen to be recommended in the most relevant category of target species. It may not be necessary to include all recommendations, provided that sufficient data from laboratory trials are supplied to cover all uses of the veterinary medicine not included in the field trials.

Wherever possible field efficacy studies should include the challenge of animals by exposure to natural infection. Unless justified, studies should compare a group of treated animals with an equivalent group of untreated or placebo treated controls.

Where the veterinary medicine under investigation is being compared with a comparator product, a group of unvaccinated or placebo treated controls should be included where possible.

Field safety studies for vaccines should be conducted at two or more different geographical sites. For non-steroidal anti-inflammatory drugs at least one field efficacy study should be performed for each claim. Field studies for veterinary medicinal products containing antimicrobial substances should be multicentric and conducted in naturally infected animals.

From the BETA 2015 survey, there are approximately 950,000 equines registered in the



UK, with the majority being pleasure horses (companion animals) with significant emotional and financial engagement. The horse racing industry turned over £229 million on on-course and £48 million off course betting in 2018. The failure to prevent disease (e.g. equine flu) or provide effective medicines (e.g. antibiotics, anti-inflammatories) would have a significant impact on the health of the UK herd, with knock-on effects on the attendance at competitions or race meetings.

The British Equestrian Trade Association's National Equestrian Survey 2015, conducted by Two Circles, found that the economic value of the equestrian sector stands at £4.3 billion of consumer spending across a wide range of goods and services each year. This has increased from £3.8 billion in 2011.

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

Procedures vary greatly depending on the study, as the majority of the work covered by this licence is commercially driven. A client provides a study outline which is assessed by a senior manager and project licence holder before a decision is made as to whether to quote for the work. This initial assessment includes an ethical aspect to determine whether the work is within the scope of the ethical policy and within the capability of the resources available. Study designs (treatment replication and animals per replicate) will be discussed with a statistician based on expected differences between treatments. All study outlines are reviewed and approved by the AWERB prior to the study starting. The project licence holder checks the study procedures against the project and personal licence authorities and is responsible for ensuring that the study outline is presented to the AWERB.

Animals will usually be screened as healthy and sero-negative for the antigens / antibodies of interest well in advance of the study starting. Females will be screened (depending on age) to confirm pregnancy status. This can be achieved by screening on their source farms or on arrival on site.

Animals may be maintained on the source farm for the study to be conducted in situ or animals may be transported to site. Immediately prior to the start of the study, animals selected for participation may be screened again to prove that they are sero-negative for the antigens / antibodies of interest at the start of the study. An inspection by a veterinary surgeon will also usually be carried out to ensure that the animals are healthy before the study commences. The vaccine / immunosera will usually be administered to a number of the animals by the recommended route and method of administration.

The administration may need to be repeated a number of times. A number of animals will usually remain untreated (or receive a placebo) and / or a number of animals may be administered a comparator product as a positive control. The positive control will usually be a product licensed for that indication in the same species. Following administration, animals will usually be sampled on a number of occasions at various time intervals relevant for the disease in order to determine response to the vaccine / immunosera. At the end of the study animals will either be euthanased, or rehomed following inspection by a vet.

Animals may be challenged with the disease the vaccine / immunosera is claimed to protect against. Challenge can either be by direct challenge to the animal or by contact with animals which have the disease (either naturally or by direct challenge themselves). The challenge material will usually be a recent field isolate and will usually have been



tested to ensure that it produces symptoms of a sufficient degree to allow efficacy to be determined. Following challenge, animals will be sampled on a number of occasions and various assessments (clinical observations, rectal temperatures, etc.) will be carried out in order to determine if the test product has been efficacious. Animals will be monitored closely in order to try to ensure that they do not exceed the protocol severity limit. This may necessitate monitoring outside of the normal working day.

At the end of the study animals will normally be rehomed following inspection by a competent person and following the rehoming policy in place for equines. However, for some studies they may be humanely euthanased to allow for collection of samples.

The number and life stage of the animal being used on study will vary dependant on the required study design. Neonates and pregnant animals may be required for studies such as vaccine efficacy and safety for registration of vaccines to provide protection during pregnancy and to neonates when first born.

The following steps/procedures will be used throughout this project:

Animals may be administered a suitable course of antibiotic on farm of origin or following transport as a prophylactic measure.

Animals may be restrained by physical means or confined within a restricted area. Animals may be housed according to the Equine Industry Welfare Guidelines Compendium for Horses, Ponies and Donkeys Administration of a sedative prior to procedures for animals which are nervous or fractious as deemed necessary by a qualified member of staff and confirmed with a vet. Sedation will always follow veterinary advice and instruction and be carried out as per sedative datasheet.

Administration of test article or control on up to 10 occasions by intramuscular, subcutaneous, intravenous, intranasal instillation, spray, orally (by gavage or stomach tube) or topically to the skin / conjunctiva.

Administration of challenge material or control on up to 10 occasions by intramuscular, subcutaneous, intravenous, intranasal instillation, spray, orally (including by gavage or stomach tube), by natural exposure (including exposure to animals which have been challenged) or topically to the skin / conjunctiva.

Veterinary or husbandry treatments may be withheld.

Blood sampling with / without the topical application of a local anaesthetic, including following non- surgical cannulation of blood vessels. A maximum of 10% of the estimated total volume on one occasion and up to 15% in a 28-day period.

Collection of samples from the nasal passage, naso-pharyngeal passage, or vagina using a swab.

Collection of faeces with a swab, faecal loop or finger.

Measurement of body temperature per rectum.

Urine collection via use of a cannula.

Animals may be killed by a schedule 1 procedure.

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



project?

This will be very varied based on the type and model of study. Depending on the life stage and disease model clinical symptoms may include weight loss, reduction in weight gain, inappetence, dullness, lethargy, behavioural changes, diarrhoea, nasal discharge, ocular discharge, vulval discharge, raised temperatures, injection site reactions etc. This list is not exhaustive and all symptoms are not applicable to all studies.

All studies will be kept to the minimum severity limit possible, with the majority being mild and some moderate. Animals are inspected a minimum of twice daily with extra obs in place when needed. Any animal with adverse affects evident will be monitored closely and treated or withdrawn from study as applicable under veterinary advice.

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)?

Based on previous PPL the estimated severity would be mild and moderate with 90% of animals at mild and 10% at moderate

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

- Killed
- Kept alive
- Rehomed

A retrospective assessment of these predicted harms will be due by 22 January 2030

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?

When testing efficacy and safety of veterinary medicines the European guidance documents require that the target species of animal is used.

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

None are applicable for registration studies

Why were they not suitable?



Target animals are required for registration studies

A retrospective assessment of replacement will be due by 22 January 2030

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?

Based on historical data from previous projects of work to develop veterinary medicine products for horses

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

Where there is a European guidance document detailing the requirements, we will comply with these. Where there is no guidance document, we will take the advice of a statistician.

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

Following EU guidance documents, sharing control groups for multiple treatment comparisons, collecting supplemental samples in addition to the primary objectives.

A retrospective assessment of reduction will be due by 22 January 2030

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 animal species, methods and models we propose to use are as dictated by European guidance documents and agreed with clients and regulatory authorities as required to comply with the regulatory needs. They will be the least invasive/harmful allowed whilst meeting the regulatory requirements.

In the majority of cases adverse effects are likely to be mild whilst a small proportion may be moderate. Where adverse effects are anticipated, animals will be monitored regularly to ensure that severity limits are not exceeded. Where severity limits might be exceeded, we will intervene to treat or euthanase the animal. Dose titration tests will be undertaken to ensure the minimum dose required is being used to reduce the severity of the challenge. Animals will be housed in guidance with current welfare guidance, ensuring adequate space, appropriate feed and the use of enrichments and lighting as applicable for the breed and age of the animal.

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

The animal species we propose to use are as dictated by European guidance documents.

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

After each study is complete they are reviewed by AWERB and any potential refinements proposed and discussed. This are then actioned by the PPL holder alongside all personnel with Home Office responsibilities to instigate throughout the establishment.

The PPL holder is the key Study Director and NTCO and is directly involved with all studies on site, constantly monitoring the progress of studies, the welfare of the animals and potential outcomes.

Technicians are in daily communication with the Study Directors and PPL Holder about welfare and health status of the animals and adjustments are made immediately as required to ensure optimum welfare and reduced severity.

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

Home Office, DEFRA and RSPCA guidance documents along with breed guides are followed to ensure optimum environments are provided for all animals. Study designs follow EU guidance documents where applicable. Studies are run to comply with GLP and GCPv regulations.

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

Through seminars, conferences, literature, sharing information with other institutes.

A retrospective assessment of refinement will be due by 22 January 2030

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. Improving treatments for blood cancer in children

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

Cancer, Therapy, Blood, Children

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 (drug treatment) and to reduce the toxicity associated with current treatments.

A retrospective assessment of these aims will be due by 26 January 2030

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 cancers, such as leukaemia, which will be better at treating the disease, improve cure rates 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 around a fifth of all childhood cancer deaths. New treatments are urgently required for children who cannot be successfully treated using current methods. This project aims to identify new treatments 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 or cellular processes that are specific to cancer cells. 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. An additional 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 blood cancer research as well as cancer research in general. 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 short-term benefits, in the long-term we envisage that this research will have a direct clinical impact within 10 years, resulting in improved outcomes for children. By demonstrating the anti- blood cancer cell properties of the drugs in mice, we will be able to develop clinical trials for their use in patients. If successful, this will result in the cure of children who do not respond to current blood cancer treatment and reduce the long term effects of any chemotherapy. 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 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: 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 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 cells grown 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 50-80% of mice in this project to experience moderate clinical symptoms, and less than 5% 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?

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 26 January 2030

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 effectiveness of drugs and experimental treatments at interfering with particular cellular pathways in blood cancer cells and eliminating them from the transplanted mice. 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 ways we have recently developed for 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 26 January 2030

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 26 January 2030

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, recent studies have 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 are 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 PREPARE 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 26 January 2030

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. Influence of fat tissue on the risk of infection and 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

Obesity, Infection, Atherosclerosis, Heart attack, Therapy

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 overall aim of this project is to understand the interplay between fat tissue and the immune system and how their function, energy storage and protection against infection are integrated. This will enable us to discover novel therapeutic ways to increase resistance to infections and treat or prevent cardiovascular diseases.

A retrospective assessment of these aims will be due by 22 January 2030

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?

Obesity and cardiovascular diseases are two main contemporary epidemiological concerns, continuously growing and having a major societal impact. Obesity rates are increasing all over the world, affecting nearly 30% of adults in the UK. The effect of obesity on increasing risk of cardiovascular diseases, diabetes and cancer is well recognised. Obesity also affects the immune system leading to increase susceptibility to infections. This has become dramatically apparent with COVID-19; obesity is one of the main risk factors of hospitalisation and death with COVID-19.

Obesity also increases the risk of pneumonia and post-operative infections, raising important clinical challenges in hospitals.

It is thus critical that we elucidate the reason behind increased susceptibility to infection in obese individuals and identify efficacious treatments restoring resistance to infections.

One of the main functions of the immune system is to protect the body against infections. An important aspect of the immune response is the production of antibodies which neutralise pathogens. Antibodies are produced by a type of white blood cells called B cells.

Some of these B cells live in fat tissues and play a very important role at the beginning of infections by producing large amounts of protective antibodies. However, our work shows that in obese individuals these B cells are lost, which leads to increase susceptibility to infection.

It is thus critical that we understand why fat tissue B cells lose their function in obesity so that we can identify therapeutic ways to restore their function. This could be in the form of a new vaccine.

Cardiovascular disease statistics (from the British Heart foundation) show that cardiovascular diseases are responsible for one in four premature deaths in the UK and was responsible for 26% of all deaths (129,147) in England in 2015.

Atherosclerosis is a disease caused by the accumulation of lipid in blood vessels causing the formation of plaques obstructing the vessels and a main cause of cardiovascular diseases. Rupture of these plaques can cause a heart attack or a stroke.

Adipose tissue B cells produce a type of antibody which protects against atherosclerosis.

Our work may lead to new treatments increasing the production of these protective antibodies to prevent or treat atherosclerosis.

Heart attacks leads to as many as 100,000 hospital admissions in the UK each year



(BHF).

After a heart attack, the body mounts a response to clear dying tissue and repair the damage to the heart. The heart is enveloped by a protective sac called the pericardium, which contains a high number of B cells. These B cells protect the heart after a heart attack increasing its repair and preserving heart function.

By identifying new pathways to boost the protective function of these B cells, we believe that this work will lead to the development of new ways of treating heart attack.

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

The major outputs we expect to have at the end of 5 years will be the discovery and validation in animal models of:

A novel approach to increase secretion of antibodies with broad anti-microbial properties which could be used in vaccines or treatments of severe infections.

A novel approach to increase secretion of antibodies which prevent or reduce the formation of atherosclerotic plaque in at risk patients.

A new pathway that stimulates the reparative properties of the immune system to increase repair of the heart after a heart attack and improve cardiac outcome.

New immune pathways involved in the regulation of adipose tissue metabolism during infection.

New immune pathways involved in the regulation of lipid metabolism which could be leveraged for the treatment of dyslipidaemia and atherosclerosis.

We would expect to publish these findings in 3-5 peer reviewed manuscripts.

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

The results from this study will contribute to advance the field of immunology and infection, by elucidating the reason why a certain type of B cells, a type of white blood cells known for their role in the production of antibodies during infection and after vaccination, are impaired in obese individuals. It will also advance the research field of cardiovascular diseases, in particular regarding the broad protective function of B cells in atherosclerosis and after a heart attack. The results from this study will be communicated through publication of papers and presentation of data to the community and will have an impact on the scientific community in the short-term (5 years) of this project.

In the long term, the results of this project will have the greatest impact in medicine. The ultimate beneficiaries of this research project will be the public and the patients suffering from obesity and cardiovascular diseases:

Obesity increases susceptibility to most infections and is a major risk factor of severe COVID-19. Identifying new drugs or vaccines that induce the production of antibodies with broad anti-microbial properties would be of exceptional value to patients and would greatly limit the burden of influenza infection and COVID-19.

Identifying new drugs/vaccines underpinning the protective effect of B cells in



atherosclerosis that could be harnessed for therapeutic benefit would also be a major advance in the treatment of patients with atherosclerosis.

While most patients survive a heart attack, the injury caused leads to adverse remodelling of the remaining myocardium and put the patients at increased risk to develop chronic heart failure.

Identifying new drugs that targets B cells to limit adverse remodelling of the myocardium and improve cardiac outcome in patients who had a recent heart attack would also be of exceptional value to patients.

During this project we will seek to develop new drugs or vaccine which if were successful in pre-clinical models we could trial in human. We believe that these new treatment(s) could significantly reduce the economic burden on the NHS within 20 years.

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

To maximise the outputs of this work we will seek to publish the results of all studies conducted under this project in where possible open access journals to ensure the widest dissemination of our research. For all publications we will use of social media accounts to share the data with the field and patient groups/charities.

For negative results we will as a minimum publish these as preprints and share the findings via our social media accounts.

Where possible we will seek to ensure we release accessible statements about our results specifically for the community.

Species and numbers of animals expected to be used.

- Mice: 5000

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.

To study the role of the immune system in infection, regulation of metabolism and repair an animal model must be used as the interplay between the components of the immune systems, the physiology underlying whole body metabolism and the pathways involved in organ repair cannot be replicated in vitro. The use of mice to study the role of the immune system in protection against infection, regulation of metabolism and repair is facilitated by (i) the availability of the mouse genome sequence, (ii) the wide availability of knockout mice, (iii) the extensive characterisation of the immune system, (iv) (v) the widest range of reagents commercially available, (vi) the relatively low cost of maintenance of mouse colonies compared to other species. Therefore, the mouse is the model of choice for our studies.

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



Below are some examples of typical experiments which will be performed: To test if a drug increases resistance to infection in obesity:

The animal is fed a high fat diet for 8 weeks to become obese.

The animal is given a substance intra-nasally to boost antibody production.

The animal is infected with influenza and disease progression is carefully monitored by recording clinical signs and weight of the animal.

The animal is humanely killed to analyse tissues and blood.

To test if a drug increases repair of the heart after heart attack:

The animal undergo surgery under anaesthesia to induce heart attack and is given analgesic.

The animal receive treatment 2 hours after surgery. The animal is carefully monitored by recording clinical signs and weight of animals during first week post-op.

The animal is lightly anaesthetised to perform echocardiography to assess heart function 21 days after surgery.

The animal is humanely killed to analyse tissues and blood.

To test the importance of a protein in a cell population for lipid metabolism:

The animal receives a substance by injection to induce a genetic modification.

Two weeks latter, the animal is gavaged with lipids and is humanely killed two hours later to analyse tissues and blood.

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

The experiments typically involve injections (e.g. in the skin between the shoulder blades of the mouse or in the abdomen). The mouse only feels momentary discomfort when it is being injected. Where this is practical, and considered less invasive than the procedure itself, we briefly anaesthetise the mice before administering any reagent (e.g. to drop it down the windpipe of the mouse).

The most severe model is pneumonia. We employ a dose of virus or bacteria at which mice will temporarily become unwell and lose weight which will last typically 48 hours before the animal start to get better and make a full recovery. However, animals carrying mutations may be worse affected. We monitor all animals throughout for adverse effects and are humanely killed should this occur.

Some experiments involve surgery under anaesthesia to induce heart attack. The animals may experience wound pain from surgery. This will be minimised by the administration of analgesics. In some rare case (<5%), the animal develops acute heart failure in the 24 hours following surgery.

Animals are thus closely monitored after surgery for signs of illness and mice showing



impaired recovery will be culled to reduce the likelihood that they will die after developing clinical signs of substantial severity. Most mice recover rapidly after surgery.

We will perform imaging for example to measure heart function using echocardiography.

This is always done under anaesthesia, therefore causing no pain or distress to the mice.

Extensive experience of animal models and a familiarity with the techniques used, allows us to recognise quickly any unexpected adverse signs. All mice will be closely monitored throughout the procedures and endpoints implemented as required.

At the end of all models used we euthanise the mice. Most of the analysis happens after that, to ensure the least adverse effects are experienced by any mice.

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 estimate the following:

70% mild

30% moderate

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

- Killed

A retrospective assessment of these predicted harms will be due by 22 January 2030

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?

There are currently no non-animal models that would allow us to study the interplay between the adipose tissue (complex organ) and the immune system (complex and multi-layered system) in various disease state (infection, obesity, atherosclerosis, after a heart attack). Therefore, our project can only be modelled using animals.

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

Our animal experiments are supported by extensive use of *ex-vivo* and *in vitro* culture systems with human tissues. We use samples of blood from donor and biopsies of adipose



tissue and pericardium obtained during the surgery of fully consented patients to specifically assess the function of human immune cells. We use next generation sequencing to characterise the normal function of these cells in tissue and the effect of obesity, heart attack, atherosclerosis etc on their function.

We will produce large numbers of antibodies *in vitro*, which will be screened *in vitro* using bacteria and virus for their antimicrobial potential.

Why were they not suitable?

The *in vitro* models give us vital information about the mechanisms we are studying but they do not allow us to evaluate the crosstalk between organ (fat tissue) and complex system (immune system) during the evolution of a disease state. Therefore, these only allow us to examine a single snapshot of a complex mechanism which may not be relevant when considered at the organism level. The entire project cannot be performed *in vitro* as to demonstrate functional effect on resistance to infection, development of atherosclerosis or cardiac function after heart attack, there are currently no viable alternatives to the use of animals.

To demonstrate that an antibody has a potent therapeutic anti-microbial potential, it must be tested in animals as there are currently no viable alternatives to the use of animals to test the efficacy of a treatment against infection. Similarly, we will produce antibodies which may have important athero- protective effect that must be tested in animal models for therapeutic potential.

A retrospective assessment of replacement will be due by 22 January 2030

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 have been calculated from previous PPL and from the planned experiments we have over the next 5 years.

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

For any study we identify the lowest appropriate group sizes for each group to give us appropriate statistical power. We use the NC3Rs experimental design assistant to help us improve the design of our experimental studies. We have robust randomisation and



blinding protocols in place to ensure we are conducting high quality studies and we publish this detail in our papers. Animal genetic backgrounds are also considered when designing experiments to ensure we are controlling variability.

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

For our experiments we only generate the number of animals we need through efficient breeding practice and we freeze down sperm when the line is no longer being used and have it available to others.

We actively use historical tissue we carefully bank when we cull animals on procedure.

This ensures we have good quality tissue and histological blocks available which allows pilot studies for new avenues of research to be conducted without the use of new animals thus reducing the number of animals we use.

For sham/naive controls we store carefully archived tissue which we use rather than new animals were possible.

The inclusion of ultrasound and MRI imaging should in future allow us to refine our ability to assess fibrotic damage in the heart, leading to a reduction of the number of animals used.

A retrospective assessment of reduction will be due by 22 January 2030

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.

Among experimental species, the mouse immune system is the best characterized, with well- established markers for defined immune cell subsets and activation stages. The methods to be employed have been developed and refined over decades of immunology and cardiovascular science research and are used in routine in laboratories studying immune responses to infections, obesity, atherosclerosis and heart attack. The use of mice is facilitated by the availability of the mouse genome sequence, the wide availability of knockout mice, the widest range of reagents commercially available, and the relatively low cost of maintenance of mouse colonies compared to other species. Therefore, mice constitute the model of choice for our study.



For the pneumonia and peritonitis models, we have made the following refinements to reduce pain, suffering, distress, or lasting harm: 1. Young adult mice will be used only to reduce mortality (compared to aged mice, older adult mice and neonatal mice); 2. Early timepoints will be taken where possible; 3. Animal husbandry to minimise suffering will include homeothermic support, fluid administration, mash, and empathetic handling.

For the coronary artery ligation model, we have made the following refinements to reduce pain, suffering, distress or lasting harm: 1. Young adult mice will be used only to reduce mortality (compared to aged mice, older adult mice and neonatal mice); 2. Early timepoints will be taken where possible; 3. Coronary artery ligation will be made at the point immediately superior to the coronary vein to standardise infarct size; 4. Post-surgical animal husbandry to minimise suffering will include homeothermic support, analgesia, fluid administration, DietGel, and empathetic handling.

Post-operative pain will be monitored and managed with analgesia. Mice will be closely monitored in the hours following surgery to ensure they are recovering normally, and those displaying impaired recovery will be killed to reduce the likelihood that they will die after developing significant physiological impairment. Mice will be observed post-operatively until moving about cage, and thereafter will be checked at regular intervals for signs of acute distress. Sham surgery will be undertaken, as this is an essential control for the method i.e. to determine levels of inflammation that are brought on by the minor tissue trauma induced by thoracotomy and suture placement. Sham surgery has no predicted mortality and is an established method in our group. In the proposed alternative less invasive electrosurgical model, a sham procedure would also be undertaken by insertion and echo guided positioning close to the heart of an electrode needle.

For substance administration we will use the most appropriate route which causes the least animal distress/suffering. For example using where possible osmotic minipumps to avoid repeated administration of substances.

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

Obesity, atherosclerosis and heart attack are disease of middle age and later life so using immature life stages would poorly model the mechanisms we seek to understand. We use terminal anaesthesia for some of our planned studies to refine our studies and decrease animal distress/suffering.

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

Regarding the infectious studies, we have refined the models we use over previous PPL projects. We use animal welfare scoring sheets for all animals undergoing infections regardless of it is asymptomatic or not. These sheets document weight, body condition, clinical signs of any discomfort and distress. This ensures that all animals are carefully monitored and ensures we maintain humane endpoints for all animals used. We will use the minimal dose of virus or bacteria required to detect a protective effect of treatments. In line with establishment policy, we will adopt the latest techniques in animal handling (e.g. cupping) to significantly reduce stress. Where possible, the least invasive methods for dosing and sampling will be applied.

Regarding the cardiac studies, we now routinely group house post-surgery which has



improved animal post-surgery condition scores. We have increased analgesia post-surgery up to 72hrs when required which has also improved animal welfare scores. We use animal welfare scoring sheets for all animals undergoing surgery regardless of it is asymptomatic or not. These sheets document weight, body condition, clinical signs of any discomfort and distress. This ensures that all animals are carefully monitored and ensures we maintain humane endpoints for all animals used.

The surgical model of heart attack by ligation of the left anterior descending artery is a long-established technique in our research centre, which has been subject to continuous refinement over 20 years. The method we will employ represents best practice in the field and is designed to minimise tissue trauma and blood loss. Intubation of animals is performed under a stereomicroscope with illumination of the throat. Successful placement of the catheter is confirmed with the observation of airflow through a water column. The appropriate region of the mouse heart is then exposed via a small opening made in the intercostal muscle between two ribs, which is gently opened with blunt-ended forceps. The ribs, sternum and pectoral muscle are not cut or damaged. The use of scalpels and sharp-ended forceps, which cause more damage to soft tissue and blood vessels, will be avoided. The artery ligation will use monofilament micro-suture with a round tapered needle in order to minimise damage to the surrounding cardiac tissue.

In addition, our research centre is currently establishing a less invasive model of heart attack than the coronary artery ligation model which significantly reduce post-procedural mortality using echography guided coagulation of the artery. If found to be suitable and reproducible, this model will be used in all instances when we don't need to study the function of the pericardium.

Anaesthesia and analgesia will be provided during the surgery and post-surgery. To reduce infection risk, the best aseptic technique will be used during surgery (eg sterilisation of instruments between animals, full surgical drapes), mice will be housed in ventilated cages. Mice will receive rigorous monitoring after surgery to observe any signs of distress.

We will measure troponin levels in plasma from tail vein at 24 hours to assess infarct size in the CAL model. If mice are found to have no infarct or an insufficient infarct, they will be killed at an earlier stage to prevent their continued use in further steps of the protocol.

Throughout, we will ensure that the least invasive methods of dosing and sampling are applied, including the use of anaesthesia for humane restraint.

For certain procedures for example blood pressure monitoring we train the animals so they are used for the procedure prior to taking measurements. This allows the animals to get used to the handling and measuring apparatus reducing distress when measurements are made.

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

We will follow the NC3R's ARRIVE guidelines to ensure experiments are conducted in the most refined way. We will consult with the named veterinary surgeons and adopt any refinements to protocols over the duration of the project.

Where possible we used published best practice guidance in our experiments. We have written SOPs which we use for training purposes and to ensure best practice.



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

To ensure that we stay informed about advances in 3Rs we will attend our university 3R's day held annually and the PiL refreshers course which updates on important new advances for example tube handling. These new advances will then be utilised during this project. We will also make use of the NC3Rs website news to ensure we are up to date.

A retrospective assessment of refinement will be due by 22 January 2030

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?



11. Pathogenesis of Heart Failure

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 Failure (HF), Redox regulation, Hypertension, Signaling, Remodeling

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

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?

Heart failure (HF) is a common clinical syndrome associated with high rates of morbidity and mortality that not only occurs in scenarios related to ischemia, infarction, diabetes, metabolic syndrome, but also during sepsis and pulmonary hypertension. The over-arching aim of our studies is to broadly investigate the role of modifications of specific proteins by reactive molecules (known as oxidants) that can form in cells and contribute to the development of HF. We anticipate that the enhanced understanding of HF that our studies should bring, will potentially allow new targets for drug development to be identified.

A retrospective assessment of these aims will be due by 21 January 2030

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?

Although HF is increasing in prevalence, there is not only a lack of effective therapies, but there also a significant deficit in our understanding of the mechanisms by which it develops. Our studies are aimed at defining the role of specific modifications of proteins in the development of HF. By undertaking such work, we enhance the likelihood of identifying novel drug targets which may ultimately result in new therapies to combat HF. HF is classified as a syndrome because it is a series of correlated symptoms or clinical signs that broadly associate with this condition, but the definite causal reasons are not well understood. As mentioned, HF, which is the inability to pump sufficient blood to meet the body's needs, occurs in multiple different situations and the causal mechanisms are circumstance dependent and their relative contributions vary with the underlying pathologies. Essentially HF is not a simple, single entity and as such it is unrealistic for any given therapy to work in all of the scenarios and forms that it can occur in. By defining the contributions of specific oxidant species to HF progression during different scenarios (e.g., ischemic heart disease, post-infarction, diabetes, metabolic syndrome, sepsis or pulmonary hypertension), this could lead to bespoke, highly targeted therapies tailored to a specific condition.

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

By the end of the research project, new genetically modified mouse lines will be created that will be invaluable in helping us understand the pathogenesis of cardiovascular conditions, especially HF that has a huge unmet need for new therapies. New knowledge and scientific information will be generated, and results that are not commercially sensitive will be submitted for publication in open-access peer-reviewed scientific journals. Ultimately, this project may lead to development of new pharmacotherapies for conditions such as hypertension, ischemic or inflammatory heart disease and HF.

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

We have a track record of bringing new insight to the mechanisms of cardiovascular health and disease, enhancing the likelihood of developing new pharmacotherapies. The studies we will perform under this licence are anticipated to provide similar new insight, some of which will relate to the identification of new drug targets and this information will be the major benefit in the short term.

Looking ahead, it is possible that we or others will identify drugs that engage these targets to offer new therapies in pre-clinical models of disease, that in the longer term after suitable safety pharmacology could lead to clinical trials in humans.

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

During the last few years, we established new collaborations with experts in the field.



Our collaborations are broad and international, and these ties will ensure wider recognition and reproducibility of our work and maximise the potential outputs arising from each study. With this, we routinely disseminate our work as new open-access submissions for publication in peer-reviewed scientific journals and present our work at national and international conferences regularly. We have numerous examples where we have published studies in which the data did not support the initial hypothesis, but it is important to share such insight with our wider community.

We are determined to share our genetically altered animals with the scientific community; therefore we will provide all our models free of charge and they will be distributed by a non-profit organisation.

Species and numbers of animals expected to be used.

- Mice: 27000
- Rats: 600

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 potential translation of preclinical work and applications in human patients crucially depends on whole organism studies in which the integrative physiology approach can be integrated into one setting. Rodents, such as mice and rats, will be employed in this work programme, as they resemble the progression of the human disease model overall. They have long served as a useful species for biomedical research animal models due to their anatomical, physiological, and genetic similarity to humans. Advantages of rodents include their small size, ease of maintenance, short life cycle, and abundant genetic resources. Genetically modified animals (i.e., primarily mice in our case, but sometimes rats) are needed as they are an ideal model to definitively establish a causal relationship between new molecular, mechanistic observations and their contribution to physiology or pathophysiology. In some cases, for mechanistic work and, crucially, for testing preclinical effects of new therapeutics, as well as studying new imaging modalities for disease prognostic and diagnostics purposes, more than one experimental model and more than one species (i.e. both mice and rats) will be studied.

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

Typically, an animal will undergo a breeding step, and once of adult age, it can be studied experimentally. Typically, the animal will undergo an experimental intervention to stress the heart and initiate progression to HF. This can be by administering drugs by mouth, by injection or by surgically implanted delivery pumps that cause high blood pressure in the arteries measured by a surgically implanted sensor, metabolic changes, or diabetes. The progression to HF will be typically monitored weekly using imaging such as echocardiography and blood sampling that involves anaesthetising the animal. How the disease progresses in a wild-type animal compared to one that is genetically altered will be mechanistically informative, as will studies in which the impact of drugs with disease- or mechanism-modifying capabilities provided orally, by injection or surgically implanted delivery pumps acutely over days or up to 3-4 weeks. Alternative ways of inducing heart



stress involve surgical approaches, for example constricting the aortic artery with a ligature to cause hypertension, or a coronary artery to cause ischemia or infarction that culminates in HF that can be temporally indexed and compared in genetically altered animals, for example using imaging techniques or blood sampling, as explained before being humanely culled and tissues collected for further analysis.

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

Our established transgenic lines typically do not develop any severe basal phenotype, but some lines demonstrate potentiated disease progression, such as HF, when subjected to stress-inducing protocols such as cardiac pressure overload or pro-inflammatory stimuli. This project licence will enable the study of novel transgenic mice that we have the funds to develop, and based on our experience they are likely only to show a pathological phenotype when they are subjected to stress interventions.

HF can be of varied aetiologies and can manifest as HF with preserved ejection fraction or with reduced ejection fraction and can affect the left and right sides of the myocardium. HF can develop slowly following hypertension or ischemia but can also occur acutely such as during sepsis. This project aims to study these various types of HF using rodents where hypertension or metabolic changes or cardiac hypertrophy or sepsis is induced by established methods some of which may involve surgical interventions. These animals are expected to develop cardiac and metabolic adverse effects including HF and/or diabetes, as an essential component of the disease process being studied.

Some of the mice will be used to study pulmonary hypertension and right heart failure using established methods including exposure to low oxygen conditions.

These animals, as with the other models of HF, will develop cardiac dysfunction but will also have an initial adverse respiratory phenotype as they acclimatise to the low oxygen conditions.

In addition, we will also study the process of vascular growth after ischemia, a key determinant of clinical outcomes in patients with cardiac ischemia and infarction. To mimic the process in different vascular beds, we will be using limb ischemia model, which can cause adverse effects including lameness of the limb subjected to ischemia.

In models that include surgical interventions, the adverse effects of surgery may include wound infection, dehydration or pain (after a few hours).

Animals assigned to one of the HF models are expected to show reduced weight gain compared to sham animals. Signs that decompensated HF is developing may include increased respiratory rates, reduced activity, piloerection, and hunched posture.

In acute rodent models of sepsis, adverse effects of a severe nature due to the immune response, alterations to haemodynamics, and organ failure will manifest as lethargy, inability to feed, hypothermia, diarrhoea (in some models) and generalised poor body condition.

In a proportion of animals used in these different disease protocols, additional interventions such as osmotic minipump implantation, telemetry, non-invasive imaging, dietary modifications, blood sampling and treatment with disease-modifying agents will be



carried out. These interventions may cause transient distress, but will be help monitor disease progression and the effect of treatments, as well as help to control the severity of adverse effects.

When injected with therapeutic or imaging agents, local tissue infection rarely may be shown by induration, acute swelling, or discharge, typically for no more than a few hours. Occasionally, after non- invasive imaging, a rapid but transient (up to 30 minutes) drop in body temperature can occur.

Throughout the project, the animals will be monitored regularly for any deviation from the expected protocol outcomes and humane end points applied to determine when the animal needs to be terminated by Sch1 methods.

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 ~10% of animals to have a severe harm phenotype, 35% of animals to have a moderate harm phenotype, ~30% with mild severity and the rest to be at the sub-threshold severity.

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

- Killed

A retrospective assessment of these predicted harms will be due by 21 January 2030

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 this research project, we aim to better understand the mechanisms of HF, a complex multi-factorial syndrome with limited therapeutic options, especially those forms involving diastolic dysfunction. This necessitates observation of chronic physiological changes within the whole organism during the various developmental changes of this condition. HF affects more than one vital organ; in men, the disease-related changes develop over several weeks to months and years. We cannot “mimic” such a condition in a dish successfully, as this will fail to provide useful information regarding how therapies may be subsequently used in humans.

Therefore, animal research and models are required to understand HF development and



its effects on body health and to test any potential therapeutic strategies in the intact animal.

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

We routinely use human and rodent cells cultured in laboratory dishes to provide initial important information before embarking on animal experiments.

We considered using vascular cell organoids, engineered tissues and/or co-culture of more than one vascular cell type, or human vascular cells derived from induced pluripotent stem cells (iPSCs). While we will still consider developing these cutting-edge approaches in the future, for the reasons highlighted above, these ultimately cannot fully replicate the in vivo course of the disease in a living system and fail to provide useful and crucial information regarding the course of the disease and how therapies may be subsequently used in humans.

Due to our ongoing collaborations, we are able to perform some complementary studies of human tissues or primary vascular cells isolated from patients. However, these are not ideal as such tissues are difficult to obtain, and available specimens are often unsuitable for research purposes in terms of quality and often come from uncontrolled populations.

It is not currently possible to use computational models, although our findings may guide future programs. In the future, artificially constructed data networks might help to predict the likely course of a disease and its response to treatment. Whenever possible, we will support our preclinical data with targeted gene-based approaches like mining exome sequencing data in the UK BioBank.

Why were they not suitable?

Cell models of hypertension, ischemic heart disease, sepsis or HF are limited in their ability to provide information that is useful in terms of translational therapies.

Although cell and other experimental models can provide crucially important information, and indeed we plan to use such complementary approaches as part of these proposed studies (which will help limit animal use), it is critically important to substantiate any findings in the setting of a more relevant model to the human disease condition.

Small animals, especially mice, are widely used in the study of the various conditions mentioned at the top of this paragraph and we aim to use these validated approaches - which we also have a lot of experience of using over many years.

Studying HF, especially the latter stages, is very difficult in test tubes or isolated cell systems – whereas the animal models we use are well-accepted, used worldwide and provide hugely valuable information relevant to humans. It is commonly accepted by biomedical experts that animal models are therefore required to fully understand the pathogenesis of complex human diseases, as well as to test novel potential therapeutic strategies that research such as ours may unveil. The mouse models we plan to use are undoubtedly directly relevant to the human HF condition, a syndrome that accounts for a high level of deaths and for which there is currently significant unmet clinical need in terms of effective, life-saving therapies.

A retrospective assessment of replacement will be due by 21 January 2030



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?

All efforts will be made to minimise animal numbers, whilst not compromising on our ability to deliver reproducible studies that the community find valuable and can have confidence in. Our experience in animal research exceeds 25 years, and this together with a multifaceted, inter-disciplinary approach to our studies that utilise complementary tools and methods, limits our reliance solely on animal and so reduces the numbers we need to use. The large number of animals required (comprising predominantly adult mice) reflects the large number of licensees (8-10) working on this research programme and the significant level of resource available to the laboratory for such studies.

Our strategy for breeding of transgenic mice involves culling a significant number of pups (comprising mainly heterozygotes) after genotyping information become available, thereby increasing the number of mice on the breeding protocol roughly 2-fold.

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

The data obtained from analysis of samples collected following terminal procedures will maximise the information derived from each subject, with tissues readily collected without causing any additional harm to the animals, which will complement our in vivo and in vitro investigations. The number of animals used will be kept to a minimum by, where possible, sharing the tissues (or isolated cells) after terminal procedures between several users. Thus, data is maximised whilst numbers are minimised as far as is practicable.

Dynamic telemetry readings and repeated non-invasive imaging enable longitudinal studies, allowing in some cases for animals to serve as their own controls. We will utilise state-of-the-art cross-sectional imaging tools in our experiments whenever possible, enabling us to track progression within an individual animal and thus maximise the data collected from each individual studied.

Various statistical programmes will be employed (e.g. GraphPad or the NC3Rs The Experimental Design Assistant: <https://www.nc3rs.org.uk/our-portfolio/experimental-design-assistant-eda>). Finally, we will also consult with the statistics specialists available within our Institute to advise on experimental design if provided with data from the pilot experiments. All experiments will be reported according to ARRIVE guidelines for reporting in vivo animal research.

What measures, apart from good experimental design, will you use to optimise the



number of animals you plan to use in your project?

All efforts will be made to minimise and restrict (reduce) animal numbers. For the in vivo experiments proposed here, dynamic blood pressure telemetry readings permit animals to serve as their own controls (compared to basal right ventricular, basal systemic or pulmonary pressure). Measuring blood pressure in animals with implantable radiotelemetric devices and employing state-of-the-art imaging techniques (e.g. echocardiography, photoacoustic ultrasound, PET or MRI) permit direct data comparison before, during and after the treatment or intervention.

Many of the animals that we plan to use will be anaesthetised and culled under anaesthetic without waking up (which does not cause any additional harm to the animals), and tissues will be collected at the end for an additional (genetic, biochemical or histological) post-mortem analysis. This will maximise the data collected from each animal used and inform further studies. Thus, data is maximised whilst animal numbers are minimised. When it is not possible to carry out these experiments without using animals, but whenever possible, we will try to avoid using live animals and instead perform experiments with isolated organs.

A retrospective assessment of reduction will be due by 21 January 2030

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.

Rats and mice are the most appropriate species to study because their genotype is easy to manipulate, blood pressure and organ function can be accurately assessed, their cardiovascular system is similar to humans and hence, have led to the development of heart failure (HF) models. We will be using female and male animals in most study protocols except where the sex-related variations will affect data collection.

Most of the studies will be carried out using mice, the use of which for this purpose offer several significant advantages. Mice are not only easy to maintain and offer a cost-effective, high-throughput model relevant with a short inter-generation time but can provide mechanistic insight - especially when specific transgenics are studied. Thus data, high in content, quality and biomedical significance, can be obtained in a timelier manner than if larger species were used.

Well-developed and clinically-relevant mouse models of HF are available and used in



many laboratories worldwide. The continued development of genetically altered (GA) animals, which we utilise routinely, can significantly aid studies of pathways related to scenarios such as HF. This allows the impact of a particular gene to be examined more precisely than can typically be achieved with the majority of pharmacological tools available. The use of genetically modified mice allows the determination of the role of genes specifically implicated in the pathogenesis of HF by preliminary investigations. This focused approach represents a refinement over investigations on wild-type mice which may not harness the power transgenic models to home in on a precise molecular pathway or mechanism. Transgenic or mutant mice will be created, because of the advantages afforded, as part of our work programme by subcontracting this to reputable, expert commercial providers.

Provision is therefore made for the breeding of transgenic or mutant animals on this project licence. Although we have chosen to primarily use mouse models of HF, we require the use of both rats and mice in some particular cases. Rats may be used as it is necessary to have a second rodent model for pharmacological studies proposed here, for example to further explore the most promising therapies following initial studies in mice. The rat heart is also much bigger, which can allow us to decrease the amount of animals needed to collect sufficient tissue, when larger amounts are needed for analytical procedures. Rat hearts are also more robust than those of mice in terms of cell isolation studies.

Over the past decades we have continually refined our in vivo and ex vivo techniques. We choose and optimise our models to provide high quality, robust data whilst impacting least on animal welfare. We employ multiple analytical techniques to determine whether our experimental interventions, involving transgenic mice or drug treatments, modulate HF progression. Many of these indices are clinically meaningful endpoints that are the same as those used in human patients with HF.

We have been developing the skills to routinely use imaging techniques such as echocardiography and magnetic resonance imaging that enable the non-invasive evaluation of cardiovascular haemodynamics and cardiac function. Longitudinal non-invasive measures of cardiac function will mean fewer animals are required to determine preclinical efficacy. Surgical implantation of telemetry probes, a method that we will routinely utilise, provides a number of advantages and considerable refinement over other more invasive surgical approaches that other groups may implement. Telemetry avoids the depressive effects of anaesthesia, which has limited terminal heart-catheter procedures. In addition, telemetric monitoring also represents a refinement because this gold standard method is well-accepted to provide a very accurate measure of blood pressure – which also decreases the data variance, allowing a reduction in the number of animals required to make robust conclusions.

Specific actions will be taken to reduce pain and suffering in the animals. We have substantial hands-on experience of the surgical techniques outlined herein, and they will be performed by skilled licensees who are or will be fully trained, and who will follow good laboratory practice, best practice of peri-operative care and consult regularly with veterinary surgeons and experienced technicians to minimize any discomfort or distress. In addition, the use of inhalational anaesthesia will be the primary method of general anaesthesia and appropriate analgesic to control will be given in a perioperative manner.

Animals will be inspected daily, or more regularly if warranted, for any signs of distress, and subjects showing significant adverse effects, or whose general health deteriorates



(e.g. hunched position, raised fur, behavioural changes, loss of appetite, reduced mobility, and failure to thrive) will be culled if appropriate by a Schedule 1 method.

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

Most of our studies will be carried out using mice, which are the least sentient small mammal model commonly available. They are relatively easy to maintain and cost effective with a short inter- generation time. Data can therefore be obtained in a timelier manner than if a larger species were used. Well-developed and clinically-relevant mouse models of HF have been developed and are routinely used in many laboratories, including ours, worldwide. The use of transgenic animals can significantly aid studies of precise pathways, for example by knocking-in or -out specific genes. This allows the impact of a particular protein to be examined more specifically than can be typically achieved if pharmacological tools alone are used. Bespoke transgenics have and will be specifically generated to aid us in our goal of better understanding the pathogenesis of HF, representing a refinement of our overall research strategy involving animals.

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

We choose our models to provide adequate data whilst impacting animal welfare the least. We intend to employ several methods to assess responses to a treatment, which are based on clinically meaningful endpoints in patients. These include longitudinal non-invasive measures by echocardiography, cardiac magnetic resonance imaging, and exercise capacity.

When studying novel agents, pilot studies on small number of animals will be carried out to ensure that the dose used in subsequent studies are effective for collecting data without causing harm and suffering in animals.

Specific actions will be taken to reduce pain and suffering in the animals. We have substantial hands- on experience in such studies, so any surgical techniques will be performed by experienced licensees who will be trained, will follow good laboratory practice best practice of peri-operative care and will consult regularly with veterinary surgeons and experienced technicians to minimise any discomfort/distress.

Inhalational anaesthesia will be the primary method of general anaesthesia, and appropriate analgesics to control pain will be given in a peri-operative manner.

Surgical implantation of the telemetry probe provides a number of advantages and considerable refinement. Monitoring is entirely remote, removing the stress of human contact whilst benefiting from continuous arterial pressure readings in the home cage.

Longitudinal physiological in vivo experiments will be followed by end-terminal procedures and in vitro analysis of harvested tissues and plasma to maximise the data yield from each experimental subject without causing any additional distress to the animals. In addition, the accuracy of telemetry monitoring, in particular, will mean that fewer animals will be required to achieve appropriate statistical power.

We have been developing the skills to routinely use imaging techniques such as echocardiography that enable the non-invasive evaluation of heart haemodynamics and cardiac function.



PhenoMaster assessment of metabolism provides a whole range of metabolic data whilst the animal is undisturbed in its cage.

Animals will be inspected regularly for any signs of distress, and subjects with significant adverse effects or whose general health deteriorates (e.g. hunched position, raised fur, behavioural changes, loss of appetite, reduced mobility, and failure to thrive) will be culled by a Schedule 1 method and the NVS will be informed.

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

We are regularly monitoring all the new guidance published on NC3Rs. New techniques and study plans are discussed with Named Animal Care and Welfare Officers (NACWOs) for the animal housing facility and the institute's Veterinary Surgeon (NVS), to ensure that the researchers are competent in carrying out the experiments in the most refined way while minimising harm to animals.

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

We have already been subscribed to the National Centre for 3Rs in Animal Research mailing list, and we receive updates about conferences, meetings, new tools or funding schemes regularly. We will continue to visit the dedicated website (<https://nc3rs.org.uk/>) and speak to our local Named Veterinary Surgeon (NVS) about new improvements and expectations. We are an active member of the local interdisciplinary Biological Services Unit (BSU) User Group, which oversees the management and day-to-day running of BSU-based equipment, the use of animals in research, training and assessment relevant to animal research, and other local issues.

A retrospective assessment of refinement will be due by 21 January 2030

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. Recovery and Repair after Nervous System 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
 - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Spinal cord injury, Traumatic brain injury and stroke, Regeneration, Neuroprotection, Therapy

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

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 develop new therapies for disorders such as spinal cord and brain injuries, which lead to severe and lifelong disability and for which there is currently no cure or any effective therapy.

A retrospective assessment of these aims will be due by 26 January 2030

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?

Injuries to the central nervous system, including spinal cord injury (SCI) and brain injuries (stroke and traumatic brain injury, TBI) can happen to anyone at any time and can be devastating, in an instant changing the lives of the individuals and their families forever. SCI and TBI typically result in serious and lifelong neurological deficits. SCI leads to paralysis and loss of sensory and motor functions, along with multiple co-morbidities, for example many patients suffer urinary, bowel and sexual dysfunction, chronic pain, and depression. TBI can cause problems with decision-making, memory and movement, and has been linked to diseases such as Alzheimer's Disease. SCI in particular imposes severe limitations on mobility, independence, full participation in society and quality of life and has an extremely high financial burden, with healthcare costs among the highest of any medical condition. SCI and TBI are recognised as the leading cause of loss of human potential around the world. The incidence of TBI and SCI is increasing, as the population ages (and more injuries are suffered in the elderly due to falls), as well as the increasing use of motor vehicles, motorcycles, and bicycles. There is no cure for these disorders and no adequate approved therapies. Patients are typically offered surgical procedures to stabilize the spine or reduce pressure on the spinal cord or brain, with no further intervention other than rehabilitation, supportive care, and management of secondary complications.

The social, health and economic impact of TBI and SCI and the lack of effective treatments makes them among the most significant global health priorities. Thus, there is an urgent need for innovative treatments that can heal and protect the nervous system after an injury. These new therapies aim to repair damaged tissues and reconnect muscles, ultimately helping to restore essential abilities like movement and sensation for individuals facing lifelong disability and paralysis. The purpose of this work is to understand the biology and pathophysiology of these conditions, to develop advanced regenerative and neuroprotective therapies, and to conduct pre-clinical work that will lead to new therapies for patients. The impact of this work for patients could mean recovering vital functions such as mobility and use of the hands (and consequently the ability to perform everyday tasks such as feed, wash, and dress independently). This work also has implications for the healthcare system and economy, although the biggest impact is the potential to improve independence and quality of life for the millions of people worldwide currently living with a lifetime of disability.

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

- New information on understanding the neurobiology of neurotrauma
- Identification of genes, proteins, molecules, signalling pathways or other factors that prevent nerve regeneration, and/or neurotoxic molecules that kill nerve cells
- Identification of new therapeutic targets, genes, proteins, molecules, signalling pathways or other factors that enable nerve growth, neuroprotection and tissue repair



- Scientific Publications (original research, review articles, white papers and policy documents) Patents and intellectual property for new therapeutics for brain and spinal cord repair
- Generate a tissue and sample biobank: collection of valuable samples (e.g. spinal cord, brain, and peripheral organ tissues, blood and cerebrospinal fluid - CSF) from longitudinal injury and treatment studies that will be available for other researchers to use
- Produce new regenerative and neuroprotective therapies that are in advanced pre-clinical stages, and/or in a developmental pathway for translating to human treatments, and/or in clinical trials
- A pipeline of small molecule, pharmaceutical and vector-based regenerative therapeutics for treating human nervous system injuries

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

Potential beneficiaries of this research are:

- The brain and spinal cord injury community; this includes patients, their families, carers, supporters, charitable foundations, donors and advocacy groups. Since there are currently no disease-modifying or regenerative therapies available for brain and spinal cord injury, new therapies that improve functional outcome would have significant health, social and economic impact. For example, recovering the ability to reach, grip, hold and pick up an object would enable autonomy (e.g. being able to wash, dress and feed independently), greater ability to participate in society, an improved sense of self-worth and consequently an improved quality of life for patients, their families and carers.
- Scientists, clinicians, neurosurgeons, patients, carers and therapists who are involved in research relating to the tissue microenvironment of the injured central nervous system, and/or how it can be manipulated to enable new connectivity and functional repair and which may lead to future regenerative therapies.
- Scientists and clinicians who are interested in rehabilitation, training and improving limb and other motor and sensory functions, and who want to understand how to best combine biological therapeutics with training/rehabilitation regimens to maximise functional recovery.
- Industry and academic partners as well as clinicians, who are interested in forming networks and consortia for dissemination of findings relating to potential regenerative therapies for SCI.
- Wider groups of researchers and clinicians interested in regenerative/ neuroplasticity/ neuroprotective therapies for improving functional outcome in many different neurological disorders, particularly disorders which affect mobility and where neuroplasticity is a potential route to restoring function (e.g. stroke, ALS, multiple sclerosis and some neurodegenerative disorders).
- Researchers and clinicians interested in small molecule, drug, gene and cell therapies for neurological disorders.
- The wider field of regenerative medicine will benefit from new knowledge, new approaches and new therapies for improving neuroplasticity, neuroprotection, regeneration, functional connectivity and meaningful recovery of motor function in the injured or diseased central nervous system.

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

- Making resources available to other researchers e.g. tissue and sample biobank



- Making our data (published and unpublished) open access to other researchers in our field using the ODC-SCI platform (the Open Data Commons for Spinal Cord Injury: <https://odc-sci.org/>)
- Dissemination of research to both scientific and lay audiences: scientific conferences, lectures, seminars, keynote talks; summer school teaching; lay talks to stakeholders and patients e.g. via our regular Spinal Research Supporters Evenings (lay talks, lab tours, meet the scientists Q&A with patients, caregivers, charities and stakeholders); school outreach and work experience students; patient and public involvement (PPI) events.
- Collaborations across different sectors, including multidisciplinary research scientists (e.g. biologists, neuroscientists, engineers, biomaterial scientists, physicists), clinicians, health care workers, patients (participatory research), industry, charitable foundations.

Species and numbers of animals expected to be used.

- Mice: 2000
- Rats: 3500

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.

Rodents (rats and mice) are an excellent model system for studies of the nervous system, and for understanding how it functions normally and how it changes in response to injury or disease. The rodent nervous system is well characterised and comparable to human in many important ways, such as their major sensory and motor pathways, their biological response to injury (e.g. similar primary and secondary injury phases) and similarities in some aspects of animal behaviour (e.g. voluntary motor function, skilled reach and grasp ability).

Importantly, using rodent models we can ensure that we create a controlled injury that has similar pathological features of human injuries and measurable impairments with which to assess new therapies, but keep these within humane limits that mean the animals will never be impaired to the level seen in humans (e.g. partial paresis in rodents, rather than full paralysis in humans) and will ensure that they retain vital abilities such as feeding, grooming, mobility.

The anatomical and physiological responses of rodents to nervous system injury and repair are also very well described, and our work can therefore build on a solid foundation of prior scientific knowledge.

In addition, mice (and increasingly rats) can be manipulated genetically, which allows us to mark specific cell populations or knock-out, knock-in, or mutate particular genes/proteins. The use of genetically altered animals will help us to identify molecules or genes that either enable or prevent tissue repair, and that can be targeted therapeutically.

For in vivo studies investigating nervous system injuries, we will use only adult rodents, to more fully understand the biological and physiological responses of the mature nervous system, and for developing new therapies that would benefit the adult population (who are



most typically affected by brain and spinal cord disorders).

For studies where we are breeding animals or taking tissues for studying ex vivo (e.g. in cell culture), we need to use rodents across the entire life span.

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

Typical surgical procedures (and severities) in this project:

Some of the animals in our project are simply being bred as transgenic mice and will not be used for any experimental procedures. Breeding is classified as “mild”, since the animals do not experience any undue suffering, apart from being held in an animal facility rather than being allowed to roam free.

Other animals are euthanised so that we can utilise their tissues for studying nervous system cells in reduced systems (e.g. cultured cells or tissue slices). This allows us to study the responses of nerve cells to regenerative therapies without causing any injury to the animal. The animals typically do not experience any undue suffering, other than occasional moderate responses to gene altering agents. Ex vivo tissue collection is therefore classified as “moderate”.

Since we are studying nervous system injury and repair, many of our animals will have to undergo a surgical procedure that causes an injury to their nervous system tissue (either their peripheral nerves, spinal cord or brain). These surgical procedures are always performed under general anaesthesia. We will use well-established models of injury, such as cutting or crushing a nerve, or a small part of the spinal cord, or a region of the brain; or a focused contusion injury (using a pneumatic device to deliver a defined impact to a small region of the brain or spinal cord); or a focused ischemic injury (temporarily blocking blood supply to a region of the brain or spinal cord). We use these injury models to understand the cellular and molecular changes that happen after injury and find out how the tissue degenerates, how the cells die, and why these tissues are unable to regenerate (unlike other tissues of the body where regeneration can be very successful). As well as trying to understand why nerve cells do not regenerate, we also want to test new treatments that will protect cells from dying, that will enable them to regenerate and to connect with their target tissues, and ultimately lead to improved neurological outcome.

Most of our injury models are classified as “moderate” since the animals have some degree of paresis (muscular weakness) in the affected limb(s). However, most of these animals recover well following injury, and impairments are often only outwardly visible when assessed on challenging tests such as walking on horizontal ladders with unevenly spaced rungs, or when trying to balance on a moving rotating platform.

One of our injury models is classified as “severe” because the animals receive a contusion injury to the upper (cervical) level of the spinal cord. These animals will typically be more impaired than animals in the “moderate” injury classification because the higher level injury means they would have some degree of paresis in their upper limbs as well as lower limbs, as well as impaired coordination and balance. Although classified as “severe” this is to reflect the early post-operative period where the animals typically will have limited mobility in the first 48 hours.

During this period, they will receive intensive post-operative provisions (including hand feeding and washing, if necessary). Following this period there is a rapid daily improvement and by 7 days post-injury animals are typically mobile and able to groom and



feed. We have many years of experience with this model and have made many refinements, including optimising the injury probe to deliver an injury that causes a sufficient impairment to be able to assess recovery of vital skilled functions, and which causes sufficient pathology to be comparable to human, but which does not leave the animal paralysed in 4 limbs. In fact, these animals typically recover so well that impairments are only outwardly visible when assessed on challenging tests such as walking on horizontal ladders with unevenly spaced rungs, when performing skilled motor movements such as reaching and grasping a food reward, or by physiological measurements of diaphragm muscle function.

This “severe” injury model is necessary for several reasons: 1) the majority of spinal injuries in humans occur at the neck level and affect hand/arm function and respiratory function, so this is the most clinically-relevant model with which to assess advanced therapies and their potential for improving outcome for human spinal cord injuries; 2) cervical level injuries allow us to look at complex behaviours such as skilled hand and arm function and respiratory/lung function (rated as a top priorities for the majority of spinal injured patients who suffer injuries at this level). It is not possible to study these vital functions in models where the injury is at a lower spinal level and only affects the lower limbs; 3) the circuits of the cervical (neck level) are more complex than those at lower spinal cord levels, since they control highly skilled voluntary movements that require many different muscle groups and many sub- classes of specialised motor neurons; 4) there are differences in the pathology of cervical contusion injuries compared to injuries at a lower spinal level in that they typically have large regions of central tissue damage as well as “tissue bridges”, and therefore require targeted therapeutic strategies that are optimised to this unique pathology.

We will only use the severe model for understanding specific functions relating to that level (e.g. upper limb/respiration) and/or if we have previously demonstrated positive effects in a moderate injury model and the next step is to find out if the promising therapeutic will translate to a more severe injury that is closer to that of a human (with similar inflammatory tissue damage). If there is no beneficial effect of a therapy in a moderate injury, it is unlikely to be of benefit in a more complex system which controls more complex functions.

Typical number of procedures and duration:

For most of our studies, animals will typically undergo 2-3 recovery surgeries, such as a nervous system injury, a therapeutic treatment, and a procedure to label groups of nerve cells so that we can visualise any new regeneration and/or new connections. They will typically be assessed throughout the study using various behavioural tests to assess sensory and motor functions, such as balance, grip strength, coordination, locomotion, and skilled reaching.

The studies typically last between 3 days (to look at effects in the acute post-injury phase) up to 3 months (to monitor recovery over time following injury and therapeutic treatment). In some instances, longer term studies are performed to test the effect of treatments in chronic injuries. These are typically 3-6 months, but occasionally can last up to 18 months. These are important and of high clinical relevance since the majority of patients with nervous system injuries are living with a chronic injury, where there is long established scar tissue, and we need to identify treatments that will be effective for this patient group.

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



Adverse events for the animals have been grouped into general, surgery-related, injury-related and treatment-related adverse events.

General

The use of anaesthetic will impact temperature homeostasis while the animal is unconscious. Depending on the anaesthetic agent used, it is likely to cause a depressed respiratory rate and decreased blood pressure. The duration of these effects is in the order of hours. Although rare, an inevitable adverse effect of anaesthesia is potential death from the anaesthetic agent.

Persistent episodes of skin biting or scratching of the skin can occur. The duration of these effects is in the order of days and typically resolves.

Some weight loss and dehydration are expected in the first few days following nervous system injury. Acute loss of body weight after surgery usually reflects loss of water. We expect a body weight loss of 10% in a three-day period and no more than 15% overall compared to the initial body weight. The duration of these effects is in the order of days.

Some adverse events are only associated with animals in long-term studies (kept >6 months). These include developing benign growths (e.g. subcutaneous fat masses) or an abdominal hernia. Both are rare (<1% of animals) and should not cause pain (vocalisation when palpated, hunched appearance, dirty/erected fur). Animals in long term studies (> 6 months) may also overgroom, causing patchy baldness around the trunk and extremities (<5%).

Surgery Related

In the initial 7 days following surgery animals (<20%) may show a reduced desire to eat and drink.

The surgery may cause transient pain to the animal (moderate severity and common occurrence). This is mitigated with analgesia and animals recover within 2-3 days. Infection at the site of the wound may occur rarely (<5%) but where it does, this may exacerbate pain. This would present as redness, soreness, heat, or swelling around a wound or skin area. With antibiotic treatment and careful husbandry this typically heals within days.

Wound closure failure may occur rarely (<5%) but will expose the animal to temporary risk of infection and tenderness around the site. Reclosing of the wound would be performed (on one occasion only), typically within 24-48 hours of the initial surgery.

A build-up of fluid may occur over the surgical site in rare instances (<5%). This usually dissipates within 2-3 days but may require aseptic drainage if impeding regular movement.

Injury Related

Conditioning injury, which is a minor and controlled injury to a nerve to study and enhance the natural healing processes, or nerve graft harvesting may result in temporary dragging of the affected limb, typically resolved by 5-7 days.

After a nerve injury, mild impairment of locomotor function, such as dragging of a hindlimb or forelimb is expected in the first 7 days and recovery of weight bearing on the affected limb is delayed into the 2nd week in most instances. Weight bearing will be recovered by 14 days post-surgery. Thereafter, animals may show eccentric paw placement and



weakness but should exhibit weight bearing on all four paws. Weight-bearing is defined as the animal displaying the ability to support its weight during stationary stance.

Spinal contusion injury is likely to have a slightly higher mortality rate than other protocols (<5% for moderate protocols and <10% for severe protocols).

After a spinal contusion, deficits are likely to be present in both hindlimbs (for thoracic level contusion) or all four limbs (for cervical level contusion). Paralysis is not complete or permanent, but some degree of limb paresis and functional impairment will be permanent. In the acute (1-3 days) stage of recovery animals may not be able to easily reach for food and water, groom normally with affected forelimbs, or move easily around the cage and typically will lay on their side during rest. While a rapid improvement in welfare typically occurs in the period of 48hrs-4 days, animals with cervical level contusion injuries are likely to continue to rest and sleep on their side during this period. In the period of 48h-5 days a rapid improvement is anticipated, though recovery to weight-bearing is sometimes delayed to the 2nd week in some instances (<10%).

Following a spinal cord contusion injury (a bruising trauma to the spinal cord) or ischemic injury (where blood flow to the spinal cord has been temporarily restricted) to the thoracic/lumbar region of the spinal cord, reflexive bladder function may be lost. Where the lesion results in disrupted bladder function, reflexive emptying is typically restored between 1-2 weeks post-injury in rats. Mice rarely recover full reflexive bladder emptying following thoracic contusion and will continue to receive manual expression of urine for the duration of the study where required. In rare cases (<5%) animals may acquire a urinary infection due to urine retention. This would present as dampness of the skin from urine around the perineum, and/or a firm or a distended bladder, and/or blood or porphyrin in the urine, and/or foul smelling or cloudy urine. These infections are treated with a course of antibiotics and typically resolve rapidly (within 1-2 days).

Rarely (<5%), a controlled cortical impact injury to the brain can provoke a seizure. Animals will display stiffness and/or jerking movements. This is most likely to occur within the first 1 hour after injury and resolves spontaneously (typically within 2 minutes).

After a contusion injury to the brain, some impairment of locomotor function is expected in the first few weeks, as measured by behavioural tests that assess balance and accuracy while stepping.

Thereafter, animals may show eccentric paw placement and weakness but should exhibit weight bearing on all four paws.

Stroke induced by photothrombosis (a localised ischemic injury created by activating a light-sensitive dye in a specific brain region) should not cause paralysis but instead will result in clumsiness of the limbs on the opposite side of the body. Feeding and drinking should be normal within two days, but gait disturbances and unilateral grooming may remain long term.

Treatment Related

Rarely (<1%) adverse events may arise from the administration of substances.

Surgical intervention or pharmacological treatment may result in persistent episodes of skin biting or scratching. The duration of these effects is in the order of days.

In rare occasions (<1%) intrathecal cannulation (a tube laid over the spinal cord to allow a slow-release delivery) may result in temporary hind limb dysfunction, or spinal asymmetry.



The duration of these effects is in the order of days and typically resolves spontaneously.

Intravenous injection into the tail vein may result in local irritation, which is typically resolved within days.

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)?

For both rats and mice, we expect the following proportions for each severity category:

Mild: 25%

Moderate: 60%

Severe: 15%

The severe category is necessary since one of our protocols (Protocol 6) more closely models the type of spinal cord injury suffered by humans. This is necessary to understand the complex biology of human injuries and to assess efficacy of new therapies prior to progressing to a human treatment. The severe classification is predominantly due to the acute post-operative period, with the vast majority of animals recovering to levels comparable to moderate within the first week post-injury.

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

- Killed

A retrospective assessment of these predicted harms will be due by 26 January 2030

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 work aims to develop novel therapeutics to promote tissue repair and recovery after injuries to the nervous system, including neurotrauma (spinal cord and brain injuries, stroke), as well as to gain mechanistic understanding of the non-resolving pathophysiology of these injuries. We also aim to develop advanced regenerative therapeutics that can promote tissue repair and restore function for these disorders, for which there are currently no adequate treatments.

For this, we need to use clinically relevant models that can mimic the complex disease pathophysiology and progression of human injuries, which evolve from acute primary injury



(immediate axonal injury), to secondary injury (ongoing inflammatory processes and tissue scarring that occur over subsequent days and weeks after neurotrauma), and to chronic injury (established scar pathology).

Current in vitro and in silico techniques are not significantly advanced to model the integrated actions of the diseased and damaged central nervous system, in particular they cannot mimic the complex inflammation and scarring processes and cellular reactions that lead to destructive tissue pathology and chronic non-resolving pathology. For this, there is no alternative to using in vivo systems, therefore mammalian animal models are required.

Rodents are the least sentient species in which studies of behavioural function and neurophysiology such as that proposed here can be conducted. They also have well-mapped anatomical and genomic characterisation, enabling study of particular neuroplasticity-, regeneration-, and neuroprotection- promoting genes and molecules. Transgenic rodents further our ability to characterise the role of specific genes and molecules in injury and repair processes.

We have a wealth of experience working with rodent models of spinal cord and brain injury, and in adhering to stringent criteria (such as ARRIVE) for these studies in terms of experimental design, reduction, and refinement.

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

We considered in vitro and reduced assays and are using them whenever we can i.e. in vitro cultures to look at extracellular matrix- immune cell interactions; co-cultures to look at neuronal-glial cell interactions; cell-derived matrix assays to look at deposition by different cell types and spot assays to look at neuronal signalling on growth inhibitory substrates.

Moreover, our work usually starts with assessing regeneration and neuroprotection in reduced models: in vitro cell cultures and ex vivo spinal cord and brain slices will be used for screening novel therapeutics for their ability to promote axonal growth, neuroprotection, cell migration, and modulation of immune cell phenotype. For these assays, we generate primary cells or tissue from a single animal for multiple experimental conditions. This enables the testing of multiple factors in a reduced system, prior to selecting only the most promising compounds for in vivo animal testing. This will reduce animal requirements as well as maximizing the gain from animal experiments.

We also use human-derived samples, such as cerebrospinal fluid (CSF) and neural tissue obtained from patients, to identify neurotoxic molecules and to assess the efficacy of potential neuroprotective therapies in cell survival and outgrowth assays.

We have also considered mathematical and computational models. For example, we are affiliated with the international Spinal Cord Injury Virtual Collective (SCIViC), a collaborative initiative designed to address key challenges in pre-clinical SCI research by creating a unified Virtual Rodent supermodel for Spinal Cord Injury (ViRSCI). This predictive supermodel will utilise advanced machine learning techniques to generate a predictive tool for evaluating the potential of emerging therapeutic strategies.

Why were they not suitable?

Although these reduced models are invaluable for mechanistic studies and for screening, unfortunately they do not recapitulate the complex in vivo scenario of neurotrauma, which



involves multiple phases of injury (acute, sub-acute, sub-chronic, chronic), multiple cell-matrix-immune interactions, multiple reactive neurobiological and molecular changes (gliosis, scarring, demyelination, cell death, inflammation, neurotoxicity, Wallerian degeneration, chromatolysis etc). For this, there is no alternative to using in vivo mammalian systems, therefore animal models are required.

We typically use the reduced models described above as a first step and then move to in vivo models for testing therapeutic efficacy in an in vivo scenario, using the least severe injury model for proof of principle studies before moving to higher burden more clinically relevant injury models.

The ViRSCI predictive supermodel is promising but is at very early stages and the AI-enhanced modelling will be based on large-scale in vivo experimental animal studies, which will also be used to validate the tool, with a predicted 5-year pipeline of development.

A retrospective assessment of replacement will be due by 26 January 2030

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 individual experiments, we think about the minimum difference between groups that we would be interested in, e.g. we want a therapy that will improve limb motor function by at least 2 points on a given scale such as the forelimb locomotor scale; or we might aim to see a 50% reduction of footslip errors on a ladderwalk task. We can then use statistical methods that help to determine the number of animals needed for a given experiment, given the minimum difference of interest.

For the licence as a whole, we can estimate the number of animals we will use based on animal use in our previous studies, and in our previous and current Licenses, the number of researchers funded in the lab and the various aims and objectives of each project (both ongoing and planned future studies).

However, as we develop new alternative approaches (e.g. an ex vivo TBI model, and AI computational models, described above), we may find that we need to use fewer animals than we are planning at present.

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



- Our work is always to PREPARE and ARRIVE guide standards (for preparing and reporting animal experiments) as minimum, with statistically powerful in vivo experimentation afforded by thorough experimental design.
- Research staff and students in our research grouping are trained in the use of experimental design platforms such as the NC3Rs Experimental Design Assistant (<https://www.nc3rs.org.uk/experimental-design-assistant-eda>), and more recently the EQIPD framework (<https://www.nature.com/articles/s41592-022-01615-y>), as well as statisticians where necessary, in order to ensure we use the minimal number of animals required to properly assess treatment effects.
- Additionally, we utilise a reporting standard developed specifically for the spinal cord injury field: Minimum Information About a Spinal Cord Injury Experiment (MIASCI), outlining transparent reporting standards for experiments using animal models of human spinal cord injury, aiming to reduce inherent bias and increase experimental value (<https://www.lembixlab.net/miasci-a-reporting-standard.html>).
- We also upload our data (published and unpublished) to an online platform where it can be used by other researchers in our field, promoting data sharing and also minimising duplication and replication of experiments, using the ODC-SCI platform: the Open Data Commons for Spinal Cord Injury. The ODC-SCI complies with the FAIR data principles to ensure that SCI data is Findable, Accessible, Interoperable and Reusable (<https://odc-sci.org/about/blog/822>).
- For all the experiments described within this project we will use a group size which is the smallest required to achieve statistically meaningful and robust results. A priori definitions of required experimental sample sizes and statistical analysis are generated based on prior experience in experiments of this kind, upon pilot studies and using power calculations based on effect sizes in previous data. For example, typically, when conducting functional studies after spinal cord injury we would use $n=10$ per group. When not assessing behavioural function (e.g., for histological or biochemical assay) we would use $n=4-8$ per group, depending on the variability of the technique and prior reported effect sizes (e.g. $n=4$ is sufficient for electron microscopy; $n=5$ for standard histological assays of lesion and scar size; $n=6$ for biochemical and proteomics assays; $n=6-8$ for anatomical tract tracing, connectivity and synaptic activity). This is based on our previously published sample size calculations and effect sizes.
- Factorial designs will be used where possible, including randomized block designs. This improves power, reduces systematic biases and allows fewer animals to be used. For example, a typical experiment will use a “factorial design” with multiple factors including injury (e.g. sham vs. spinal cord injury), treatment (e.g. control vs. therapy), rehabilitation group (e.g. no rehab vs. rehab). A “randomized block design” is often used in conjunction with appropriate analyses that account for random factors: animals are assigned at random to “blocks” for operations and/or for behavioural assessment. This controls for the potentially confounding effect of surgery session or behavioural testing session.
- Wherever appropriate, we would also use crossover designs where the same experimental unit (the rat) is tested with multiple pharmacological agents in their ability to promote recovery of function, particularly if assessing recovery from chronic injury when behavioural function has reached plateau.
- We will also seek to reduce the number of animals studied by careful experimental design, the adoption of sensitive outcome measures with small variation and the study of only the most relevant time points.
- When using spinal cord and brain injury experimental models “sham” animals are frequently included which involve spinal cord exposure (laminectomy) only. Sham animals are important for ensuring that we have accurate baseline control data, where we can be sure that biological effects are due to the injury and/or treatment rather than



low levels of inflammation and gliosis that can occur due to the laminectomy/craniotomy procedures required for exposing the spinal cord/brain. However, our extensive in vivo experience means we can often utilise historical data, without generating additional sham animals for new functional studies.

- For biochemical/molecular biology analysis we also have a "bank" of naive and sham protein and mRNA isolates meaning additional controls do not need to be generated alongside each experiment, reducing animal use.

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

- We use effective breeding programs to limit unwanted production of offspring.
- We aim to use each experimental animal for multiple outcome measures, thereby reducing the number of animals used, and improving the power of the study (allowing correlations between outcome measures on a per-animal basis). For example, multiple functional outcome measures (behavioural testing and neurophysiology) are used to assess recovery over time, and in the same animal we will take multiple tissues at the study endpoint to analyse anatomical and biochemical changes.
- As a laboratory which specialises in in vivo central nervous system injury models, and where reduction is not always possible, we actively strive to retain all potentially useful tissues and samples from each animal and disseminate these tissues to others where it is not specifically required by us. For example, we keep tissue from the entire neuroaxis (entire brain and spinal cord), regardless of which brain area or spinal segment has been injured and/or treated. We also keep peripheral tissues and organs such as muscle, skin and sensory ganglia, lung, spleen, liver, bladder, urethra). We also keep blood and cerebrospinal fluid samples, typically at a terminal time points but occasionally at selected longitudinal time points particularly for long treatment studies. These samples will either be used for research studies conducted by our lab, but often will be shared with other research groups. Additionally, if an animal must be culled prior to a planned study end (e.g. if it has reached a humane end point limit), multiple tissues will be harvested (as above) and used for early assessments e.g. of gene expression and biodistribution (no animals will be wasted). Thus, we will maximise the data that can be obtained from each individual experimental animal.
- We also subscribe to data-sharing platforms in the field, which are designed to enable bioinformatic pooling of animal datasets across multiple laboratories and cross analysis - again reducing the number of animal experiments required.
- Pilot Studies: For therapeutic treatment studies, we will typically perform small pilot studies with sub-acute time points (1-2 weeks post-injury) for early confirmation of in vivo efficacy, prior to carrying out a long-term functional study. For example, a 2-week study in uninjured animals may be performed to confirm efficient biodistribution and in vivo transduction of a gene therapy in brain and spinal tissues prior to carrying out long-term functional efficacy studies in brain and spinal cord injury models. Similarly, to determine if any treatment is neuroprotective after injury, we would first perform a small study with an early time point (7 days post injury) to evaluate early indications of reduced gliosis, necrosis and cavitation. This allows us to make stop/go decisions with as few animals as possible. For successful pilot studies, we would then proceed with a longer-term study looking at efficacy in improving physiology and functional neurological outcome.
- Experimental Neurotrauma Biobank: we are in the early stages of building an Experimental Neurotrauma Biobank. We have started collecting and archiving tissues and samples from experimental animals, particularly when the animals have been under a severe protocol and from chronic studies. Since there are few labs worldwide



with the skills and expertise to work with complex clinically relevant neurotrauma models, we are frequently asked to share our data and tissues. Thus, we have begun to archive tissues and samples, with a view to sharing these with the community. For example, there are groups in the USA who are interested in analysing peripheral organs (e.g. lung, spleen) after spinal cord injury, and we are sharing tissues with them that we have collected over acute to chronic post-injury time points. This Experimental Neurotrauma Biobank will be an important resource for our field, making available to other researchers valuable tissues and samples from spinal cord and brain injured experimental animals, avoiding duplication, reducing unnecessary animal studies, and ensuring we make maximal use of every single experimental animal under the License.

A retrospective assessment of reduction will be due by 26 January 2030

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.

Since the overall aim of our project is to develop new therapies for spinal cord and brain injuries, we will use in vivo rodent models of nervous system injury, including peripheral nervous system injury, spinal cord injury, traumatic brain injury and stroke.

For every experiment, we ensure that we choose the model that causes the least pain and suffering to the animals, while still being scientifically appropriate.

Specifically, we will use lower force contusions wherever possible, and will use lower segmental level injuries (thoracic) prior to a higher level (cervical) injury as well as use injury models that cause paresis in only one limb or one side wherever possible.

For peripheral nerve injuries, we will injure the minimum number of nerves to be able to answer our scientific question (e.g. we can evaluate regeneration in an individual nerve in the hindlimb, without injuring all the nerves that innervate the leg and foot).

For brain and spinal contusion injuries, we will select the impact force and probe size that will produce injuries which result in functional deficit and partial paresis, but which do not cause extensive pain or complete loss of limb function, and we provide extensive provisions in post-operative care.

The nature of our research requires us to work with one severe model, since we are aiming to develop advanced regenerative therapies that will eventually be translated into clinical treatments for patients suffering from debilitating spinal cord and brain injuries.



For example, the majority of human spinal cord injuries occur at the cervical/neck level as a result of a contusive impact. Therefore, we will use a model of cervical contusion (bruising injury to the spinal cord delivered using a force-controlled impactor device) in adult rats for (i) high clinical relevance, (ii) to study the circuitry and pathology of lesions at this level and (iii) to test the potential of our regenerative therapies to recover functions of high priority to patients (arm and hand function, diaphragm function). These evaluations can only be carried out in cervical injury models because the anatomical and physiological circuits of the cervical spinal cord are more complex than those at lower spinal cord levels, since they control highly skilled voluntary movements that require many different muscle groups and many sub-classes of specialised motor neurons. There are also several differences in the pathology of cervical contusion injuries compared to injuries at a lower spinal level in that they typically have large regions of central tissue damage and inflammatory pathology, as well as residual tissue bridges, and therefore require targeted therapeutic strategies that are optimised to this unique pathology. We will only use the severe model for evaluating a candidate therapy if we have robust data indicating efficacy in less severe models (if there is no beneficial effect of a therapy in a moderate injury, it is unlikely to be of benefit in a more complex system which controls more complex functions), and/or for assessing vital functions such as digit dexterity, respiratory/diaphragm function. These studies will be essential for translating our experimental therapies to clinical treatments for SCI.

We have made significant refinements to these injury protocols, in particular the contusion injury model, over a period of 14+ years. These refinements include:

- Optimising the injury probe (with differing probe size and dwell time parameters set for injuries at different spinal levels) and injury force (with set boundaries for injuries at different spinal cord levels) in order to deliver an injury that causes a sufficient impairment to be able to assess recovery of vital skilled functions, and which causes sufficient pathology to be comparable to human, but which does these within humane limits that mean the animals will never be impaired to the level seen in humans. For example, the injury forces we use induce paresis not paralysis, enabling animals to feed, groom and ambulate while leaving sufficient functional deficits with which to measure recovery (e.g. lack of skilled grasping ability, gait irregularity when stepping).
- Developing a series of SOPs for post-operative care that include many refinements, such as intensive post-operative provisions within the acute post-injury period (including hand feeding with specialised foods and supplements, and bathing the animals where required; recovery in a post-operative incubator as standard).
- Using animals of a higher weight (>250g) for these models wherever possible, as they tolerate the injury and show more rapid recovery than smaller animals.
- Using inhalation anaesthesia wherever possible, as this leads to more rapid recovery than with injectable anaesthetics.

These recommendations for refinements, and many of our standard operating procedures (SOPs), have been used in best practice documents for improving animal welfare in experimental spinal cord injury models. We will take great care to continue refining these models and to ensure the highest standards of animal welfare throughout the Project.

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

While spinal cord injuries can be modelled in less sentient species such as zebrafish, these species have very different cell, molecular and immune responses to mammalian



species. In fact, the zebrafish, particularly larval zebrafish, can successfully fully regenerate their spinal cords after a complete spinal transection injury and so are not comparable to humans in terms of their injury and repair response. Thus, they are typically used to identify pro-regeneration genes, molecules and mechanisms. However, to test mechanisms of regeneration failure in mammals, and/or whether a potential therapeutic approach will promote tissue repair and functional recovery in a pre-clinical model that will translate to benefit in humans, a mammalian model is required. In particular, a model that mimics the injury pathophysiology and progression such as a rodent contusion injury. Rodents are the least sentient species in which complex studies of behavioural function and neurophysiology such as that proposed here can be conducted.

Additionally, one of our priorities is restoring functions that are of high priority to humans living with spinal cord and brain injuries, for example recovering use of the hands. Rodents are the least sentient species where skilled voluntary motor behaviours such as hand shaping, and reach and grasp can be modelled, for example the coordinated sequence of movements required for reaching and grasping an object are remarkably similar in rats and humans. Thus, rodents are the least sentient species where complex motor behaviours akin to those of humans can be studied and the most appropriate and optimal species for the studies proposed in this project.

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

For studies involving recovery surgeries, we will use the following refinements:

- Peri and post-operative analgesia will be used alongside effective anaesthetic regimes
- Isoflurane anaesthesia is preferred over injectable anaesthetics (such as ketamine) for rapid recovery, and will be used wherever possible
- During recovery from anaesthetic, animals will be placed in a humidified incubator
- Animals will typically be housed communally unless implanted devices necessitate single- housing (<10% of animals in this project will require single housing). In these instances, animals will undergo regular handling and enrichment
- Where a modified diet is provided (such as soft food, hydrogels), this will be pre-introduced to prevent neophobia
- For some studies we utilise a contusion injury model, either to the thoracic region, or a more severe injury to the cervical region (the majority of human spinal cord injuries occur at this spinal level). We have extensive experience with the contusion injury model (14+ years) and over this time, and with guidance from the NVS and Home Office Inspectors, we have made many refinements to this model, as follows:
 - We have carefully calculated the specific parameters (such as the minimum injury force and probe size) to create injuries that cause paresis but not paralysis, enabling animals to feed, groom and ambulate while leaving sufficient functional deficits with which to measure recovery with our therapeutic treatment. For contusion injuries at the thoracic level, animals will recover overnight in cages with modified absorbent lining and the cage will be placed in a recovery holding incubator for at least 24 hours. To ensure food and hydration is always accessible, caging provisions include easily reachable long water nozzles and easily accessible food added to the cage floor in addition to hoppers, as well as provision of hydrogel. Animals will undergo manual bladder expression twice daily until reflexive emptying is restored (typically within the first 7 days post-injury).
 - For cervical contusion injury, animals will recover overnight in cages with modified absorbent lining and the cage placed in a recovery holding incubator for at least 48



hours. To ensure food and hydration is always accessible, caging provisions include easily reachable long water nozzles, plus hydration gel and modified diet (e.g. crushed peanuts, soft chow) will be added to the cage floor. Soft bedding and sawdust will be added in increasing quantities alongside absorbent cage-liner over the following 2-7 days to maximise ability to locomote in an accessible but enriched environment. In the 1-3 days post-operatively, animals will be closely monitored, given subcutaneous saline, bathed if grooming is insufficient, and hand-fed if necessary. We typically hand feed rats with a syringe (nutritional diet recovery gels or peanut butter mixed with water) several times a day for the first 72 hours, beginning on the day following surgery. We have found these provisions to be significantly beneficial for improving general health and welfare of these animals and for more rapid recovery in the acute post-injury phase.

- We have established that the weight and strain of a rat is important when varying the strength of the injury. Wherever possible we will use Lister-Hooded rats for cervical contusion injuries as our experience has found that they tolerate this injury better than rats of other strains and they have greater activity and interest in grooming, food and hydration following surgery. For cervical contusions, we have established the minimum weight size that will well tolerate the injury (e.g. rats that are equal to, or greater than >220g tolerate cervical contusion injuries and recover well).
- With guidance from the NVS, we have refined the methods and routes of dosing of contused animals with pharmacological agents that induce gene expression. For example, we have optimised an injectable drug formulae because oral gavage positioning/ scruffing is not optimal in animals following surgery to the cervical/neck region.
- Even with our most severe injury models, we will strive to reduce the suffering of animals from severe to moderate wherever possible by ongoing refinement of our procedures in consultation with others. For example, we have produced a detailed monitoring and post-operative care protocol, which we aim to regularly update as we continue to make refinements to this Protocol.

Of note, our cervical contusion post-operative care protocol forms part of a policy document for Ethical Research for Spinal Cord Injury, which aims to facilitate implementation of the three Rs, reduce experimental variability and increase translatability within this critically important research field.

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

We will be using the EQIPD guidelines for our experimental design (<https://www.nature.com/articles/s41592-022-01615-y>) and the ARRIVE 2.0 guidelines for reporting.

We are also relying on:

Guidelines for refining administration of substances:

<https://journals.sagepub.com/doi/10.1258/0023677011911345>

The Laboratory Animal Science Association Guidelines for Aseptic surgery:

https://www.lasa.co.uk/PDF/LASA_Guiding_Principles_Aseptic_Surgery_2010.2.pdf

Guidelines for welfare assessments:

<https://journals.sagepub.com/doi/10.1258/la.2010.010031>

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



Our researchers regularly access the NC3R website to see their latest news and check on their resource library. They are also encouraged to attend NC3Rs workshops, for example the Experimental Design Assistant Workshop and the Pan-London 3Rs Symposium.

We also keep abreast of any new NC3R-relevant technologies through collaborators and/or scientists at conferences and through publications.

We continually work with collaborators and other experts in our field to share expertise and experience with refining experimental injury models. For example, several of our Principal Investigators belong to expert working groups for ethical research for spinal cord injury and have worked in collaboration with the RSPCA to facilitate the implementation of the three Rs, reduce experimental variability and increase translatability within this critically important research field. We have also made our post-operative care protocols available to other researchers, and have shared our experience with refining these protocols, to ensure best practice for animal welfare in this research area.

We will discuss new advances in our regular team meetings and seek training where necessary before implementing them.

A retrospective assessment of refinement will be due by 26 January 2030

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?



13. Sensory processing, learning and cognition in adaptive and maladaptive behaviours

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

Anxiety, Stress, Neurophysiology, Navigation, Visual system

Animal types	Life stages
Mice	adult, embryo, 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.

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?

This project aims to determine how sensory information is processed across the neural circuits that generate cognitive representations of the external world, in adaptive and maladaptive behaviours, focusing on the development of stress-induced anxiety.

A retrospective assessment of these aims will be due by 25 January 2030

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?

Anxiety disorders are a critical cause of disability, afflicting 18% of adults with a lifetime prevalence of more than 28%. Anxiety disorders constitute a major worldwide health problem, but the circuit mechanisms that define these pathologies remain largely unknown. Here, we aim to characterise the neural circuits underlying the development of stress-induced anxiety. We focus on the roles that emotional distress plays in sensory processing, perception, navigation, and cognition (“Sensory processing”: the process that organizes and distinguishes sensory information from one's own body and the environment, thus making it possible to use the body effectively within the environment).

This project will yield novel insights regarding the cellular and circuit underpinnings of adaptive and maladaptive behaviours and chronic stress (“Maladaptive behaviours”: behaviours that are, or have become, more harmful than helpful, in contrast with adaptive ones, which are more helpful than harmful). This project will potentially provide new therapeutic targets for future pharmacological intervention.

Our research will significantly benefit the clinical and academic community interested in the neural circuits underlying anxiety disorders and behavioural avoidance (“Behavioural avoidance”: any act or series of actions that enables an individual to avoid or anticipate unpleasant or painful situations, stimuli, or events, including aversive stimuli). Systems neuroscientists actively study the circuit mechanisms mediating “physiological” anxiety, namely the adaptive state of increased apprehension that helps animals avoid potential danger. We first must understand the logic of the neural networks that support normal anxiety to develop better treatment for the inappropriate expression of pathological anxiety.

This project will cover both states of physiological and maladaptive anxiety, following the changes in brain activity during the development of stress-induced anxiety (“Stress-induced anxiety”: the condition in which a big event or a buildup of smaller stressful life situations may trigger excessive anxiety).

Because of its translational relevance, this approach will generate new knowledge that will benefit clinical and basic scientists across different research fields (“Translational research”: the research aimed at translating - converting - results in basic research into results that directly benefit humans). By characterising the neural activity and defining the causal roles of specific brain areas in the pathogenesis of stress-induced anxiety, our results will especially benefit clinical scientists searching for new pathogenetic biomarkers (“Pathogenesis”: the cause and development of a disease. “Biomarker”: a measurable indicator of the severity or presence of some disease state)

Our research will also benefit circuit neuroscientists interested in spatial navigation and natural behaviours. This project will generate new model systems for studying the mechanisms that modulate defensive and avoidance behaviours.



Finally, the proposed research will benefit researchers in visual neuroscience and the academic community that studies the mechanisms of visual processing and sensory perception. Our research will increase our understanding of how vision works in a clinical condition known as “blindsight”. People who suffer from this condition are considered clinically blind because of damage to the part of the brain primarily involved in processing visual information (i.e. “the primary visual cortex”).

These patients, even unconsciously, can still identify the position of incoming threatening objects. This phenomenon is believed to depend on visual pathways independent from the primary visual cortex (“Visual cortex: the area of the cerebral cortex of the brain that processes visual information). Our work focused on the parallel visual pathways (i.e., “routes”) that connect the eye to the brain will, therefore, also benefit the medical community that studies blindsight in human subjects.

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

The outputs generated by this study will mainly be behavioural, physiological, and imaging quantitative data. These outputs will shed light on the intricate neural circuits implicated in stress-induced anxiety, with a particular focus on the interplay between emotional distress and sensory processing.

Behavioral Data: Detailed analyses of animal behaviour in response to external stimuli and stressors, providing insights into adaptive and maladaptive responses to environmental stimuli.

Physiological Measurements: Examination of physiological markers associated with stress-induced anxiety, (such as plasma cortisol/corticosterone levels, or neural electrical activity), offering quantitative assessments of stress responses.

Imaging and Electrophysiological Data: Recordings elucidating changes in brain activity and connectivity patterns associated with anxiety-related phenotypes.

Quantitative Data: Quantitative analyses of neural activity and circuit function, offering numerical assessments of neuronal responses and synaptic plasticity.

By generating a comprehensive dataset encompassing behavioural, physiological, and imaging data, we aim to provide a holistic understanding of the neural mechanisms underlying stress-induced anxiety. By comparing animal models to human data and evaluating their fidelity in representing the human condition, we aim to establish robust experimental paradigms for investigating anxiety-related pathologies.

These research outputs will be used to produce new scientific publications, and will be presented at national and international conferences and meetings. These research outputs will offer potential avenues for the development of novel pharmacological interventions targeting anxiety-related pathologies.

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

Our research outputs will contribute to the understanding of the neural circuits underlying anxiety disorders and behavioural avoidance, which will benefit both the clinical and academic communities.



Currently, systems neuroscientists study the circuit mechanisms that mediate physiological anxiety, which is the adaptive state of increased apprehension that helps animals avoid potential danger. To develop better treatment for pathological anxiety, we first need to understand the logic of the neural networks that support normal anxiety. Thus, our research will cover both states of physiological and maladaptive anxiety. By characterizing the neural activity and defining the causal roles of specific brain areas in the pathogenesis of stress-induced anxiety, our results will significantly benefit clinical scientists who are searching for new pathogenetic biomarkers (i.e., a biological indicator that provides information about the underlying mechanisms or causes of a disease or condition). By investigating the mechanisms of physiological and pathological anxiety and testing pharmacological agents in a controlled experimental environment, we will contribute to the development of new treatments for anxiety disorders. Our work has the potential to advance the field of neuroscience and provide a better understanding of the brain mechanisms involved in anxiety, ultimately benefiting both patients and healthcare providers.

Our research is also expected to have a wide range of benefits for researchers in visual neuroscience and spatial navigation. This project builds upon our recent discovery of a new "primary visual cortex", a brain area at the interface of the visual and spatial navigation systems. This discovery has raised numerous novel questions in the field. The results of the proposed research will be of great interest to the academic community that studies the mechanisms of visual processing, sensory perception and spatial navigation.

Finally, the proposed research will also be highly valuable to circuit neuroscientists interested in the flexibility of natural behaviours ("Behavioral flexibility": the adaptive change in an animal's behaviour in response to changes in the external or internal environment). The project is expected to generate new model systems that can be used to study the mechanisms that modulate defensive and avoidance responses, thus contributing to a deeper understanding of the neural circuits that underlie these complex and fundamental behaviours ("Avoidance response": a behavioural response that prevents an aversive stimulus from occurring).

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

All published data and data suitable for open access will be made freely available and accessible to anyone via our lab data repository. To maximize the use of software and analytical tools developed in our laboratory, we will upload our code to public repositories (Github) and annotate it appropriately to ensure others can use it.

Our chronic electrophysiological and two-photon imaging recordings (i.e., techniques that allow the study of the responses of neural circuits in real-time) will generate large databases suitable for sharing. To make the dataset easily accessible to others, we will use well-parametrized stimuli, compatible and in line with the Allen Brain Atlas (<http://observatory.brain-map.org/visualcoding>).

Raw and processed data will be made available at the time of publication and accessible to anyone upon request. In addition, if the journal offers the option, the acquired data will be made available as source data sets associated with the published papers. During the development of the project, we will share our analysis code through public repositories.

These data will be presented at national and international conferences and meetings, and disseminated through F1000Research.



Species and numbers of animals expected to be used.

- Mice: 9120

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.

This project includes research on adult transgenic mice. We use mice because they are the lowest species that provide adequate physiological, anatomical and behavioural repertoires for the proposed research. The complexity of the processes described in this study (e.g., the development of stress-induced anxiety) cannot be duplicated or modelled in simpler systems. Investigating the neural circuit mechanisms that underlie cognitive processes and memory consolidation necessitates using a living animal that can freely behave.

Simplified experimental settings like tissue culture or artificial computer simulations cannot adequately explore the behavioural functions of the mammalian brain. Techniques commonly employed in humans, such as functional brain imaging, have spatial and temporal resolution limitations.

Furthermore, they only provide correlative information about the relationship between certain brain structures and their function, without addressing the crucial question of causality ("Causality": the study of how things influence one other, how causes lead to effects). While studies on human patients with neurological damage can potentially offer causative insights, they lack neuronal or circuit specificity since the damage is often uncontrolled and rarely specific to precisely identified neural circuits. Given that substantial evidence demonstrates similarities in the normal functioning of various brain regions across humans and rodents, it becomes possible to extrapolate findings from one species to another ("Extrapolate": to guess or think about what might happen using information that is already known).

Mice are specifically selected as the model organism for this project due to their exceptional translational applications. "Translatability" refers to the likelihood that findings from the model organism can be directly applied or extended to human systems. Several factors contribute to the translatability of mice in this context:

Physiological Analogies: Mice share significant physiological similarities with humans, such as the general physiology of the brain and the stress responses. This close alignment enhances the probability that physiological responses observed in mice can be extrapolated to humans.

Anatomical Correspondence: The anatomical structures of mice closely resemble those of humans, particularly in the organization of the nervous system, brain regions, and neural circuits. This anatomical similarity enhances the relevance of studying cognitive processes and memory consolidation in mice for understanding similar processes in humans.

Behavioural Similarities: Mice exhibit a behavioural repertoire that includes social interactions, learning, avoidance, fear and memory - essential components for studying



cognitive processes. The behavioural similarities between mice and humans make it more likely that observations in mice can be translated to human behaviour.

Complex Process Representation: The complexity of the processes under investigation, such as stress-induced anxiety and memory consolidation, requires a model organism that adequately represents these intricacies. With their advanced behavioural and cognitive capabilities, mice provide a model system that closely mirrors the complexity of these processes in humans.

Translational Value: The findings from studies using mice are often more translatable to humans than those using more distant species. This high translational value is essential for bridging the gap between preclinical research and potential human applications, particularly in stress-induced anxiety and cognitive processes.

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

This project focuses on sensory processing, learning and cognition, and studies how activity in brain cells is linked to adaptive and maladaptive behaviours, in control conditions and under stress. It will be, therefore, necessary to perform surgeries to implant the experimental animals with recording devices, monitor their brain activity and behaviours, and reliably induce a measurable state of stress.

During the surgery, while the mouse is under anaesthesia, we will implant a head post.

This device is a small piece of metal placed on the skull that can be connected to an external holder to fix the position of the head during behavioural testing. Using a head post is the best way to fix the head without causing pain compared to clamping the animal's head directly. Fixing the head is necessary for the majority of experiments to be able to control the sensory information that the mouse receives.

Fixing the head is also necessary to allow for activity measurements in brain cells using microscopy. During the surgery, we additionally make small openings in the skull (i.e., "Craniotomies") to gain access to the brain and allow for neural activity measurements. In the opening, we can insert electrodes in the brain to measure the electrical activity of neurons or inject substances that label brain cells, enabling us to measure cell activity optically using microscopy in later experiments. In some mice, we inject a substance to make some brain cells sensitive to a specific light or implant a small tube in the brain. In a later experiment, this allows us to use either light stimulation or add a substance in the brain via the tube to temporarily increase or decrease activity in some brain cells. We use this strategy to test what function those cells have in selecting sensory information. At the end of the surgery, the openings in the skull are sealed with either a transparent glass window to allow for microscopy or with a special type of sealant.

Mice recover within a few days and are given painkillers and post-operative care (for example, they receive special food that requires minimal chewing). After the mice are fully recovered, we train them in behavioural tasks. Mice participate in experiments where they learn new associations between sensory features, mildly aversive stimuli, and rewards.

Mouse behaviours will be monitored either in freely-moving conditions (e.g., with the animals freely exploring open arenas while foraging for food) or in head-fixed conditions (i.e., the head of the mouse is fixed using a head post). During head fixation, we also place the mouse on a treadmill. The treadmill allows the mouse to move more freely (apart from



the head, which is fixed) because the mouse can choose to either sit still or walk (the treadmill only moves when the mouse decides to move) instead of being forced to sit still without a treadmill. Mice become accustomed to head fixation within a few days, and the treadmill helps mice get used to head fixation more quickly than without a treadmill. Mice will then be presented with sensory features, including visual objects on a screen, sounds from a speaker, or smells coming from a tube. Mice learn within several days which features are associated with food rewards. For example, mice learn that when they see one type of visual pattern (e.g. vertical stripes), they can get a food reward when they lick a spout and that they cannot get a food reward when they see another visual pattern (e.g. horizontal stripes).

Mild aversive stimuli (e.g., escapable shocks) and rewards can be provided in specific contexts (e.g., in the presence of specific visual cues). These mild (i.e., low amperage) shocks are escapable and triggered by specific actions of the mouse, so the animal can decide whether to trigger or avoid them, depending on the context of the paradigm. For example, mice learn that when they see one type of visual pattern (e.g. oblique stripes), they can avoid the mild shock if they start running on the treadmill right after the presentation of the visual stimulus (the speed of the running wheel is constantly monitored by a dedicated computer that controls the delivery of the electric shock). The intensity (i.e., amperage) of the escapable shocks is the minimal sufficient stimulation required to induce behavioural and neuronal effects: the perceived intensity is similar to the tingle felt during a static shock. After the mice have properly learned the tasks, imaging and electrophysiology recordings will be performed, for example, through a transparent cranial window previously implanted or through silicon probes inserted after the mice have reached the appropriate behavioural performance. Sometimes, we need to restrict the food of the mouse to motivate the animal to learn a new task. Food-restricted mice are more engaged in the behavioural task because they appreciate the food reward received more when the task is successfully executed. To avoid the animals experiencing discomfort, we closely monitor the mice and check their weight to ensure they stay healthy. In some of our experiments, head fixation is not needed, and animals learn the associations between sensory features and food rewards while freely moving inside a training cage in which visual features, sounds and smells are presented to the animal. In these experiments, we usually attach temporarily lightweight sensors to the head post in order to be able to measure the behaviour of the mouse (for example, the position of the animal in the training cage and the eye position of the mouse by using a miniature camera). We found that mice are not bothered by these lightweight sensors; for example, the sensors do not change how mice move around. We also study natural behaviours that do not require training or food deprivation, such as exploration of new environments.

After all experiments are completed, mice will be killed by a humane method. Their brains will be studied to obtain additional details about the brain cells that were recorded, including their location and cell type.

Severe harms

Since one of our key goals of this project is to study the development of stress-induced anxiety, a small subset of mice will undergo stress-inducing protocols in which they will be exposed to inescapable electric shocks. These protocols are used to induce a severe state of stress in the animal, and are therefore considered as "severe" procedures. In this phase, the animal experience entails the controlled administration of inescapable electric stimuli designed to elicit a stress response.

During the sessions, electric shocks are applied through a grid of evenly spaced



conductive metal rods, typically targeting specific areas like the feet. The calibration of shocks is meticulously carried out in accordance with the experimental design, considering factors such as intensity, duration, and frequency. The intensity and numbers of the stimulations will be the minimal sufficient required to induce behavioural and neuronal effects: the mice will not display any overall clinical manifestations, but rather show specific alterations in behaviours within specific task conditions. The number, magnitude (amperage) and duration of the protocols are kept to a minimum for both welfare and scientific reasons (i.e. to avoid non-specific suppression of behaviour). It is critical for our experiments that the effects of the stressors are carefully monitored both at the behavioural and neural levels. This precise monitoring allows us to gather the data necessary for addressing our scientific aims. In addition, it enables us to refine the stress-inducing protocol in such a way that the intensity of the stimulations is minimal enough to induce measurable behavioural and neuronal effects.

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

Most expected adverse effects are associated with the initial acute recovery phase after surgery. We expect all animals undergoing surgery to make a rapid recovery post-surgery.

During this phase, complications may arise from the surgical procedure, such as localised swelling, or from the prolonged use of anaesthesia, such as a delayed return to normal behaviour. These mild clinical signs typically resolve within a few hours after surgery, but in some cases, they may extend up to approximately one day after surgery. Control measures will include the provision of mash or recovery gels post-surgery and the provision of additional warmth. By following best practices, including providing a comprehensive analgesic regimen and closely monitoring the animal, the overall impact of surgery on the animal is minimised as much as possible. The severity of surgical procedures will be minimised by applying pre- and post-operative analgesia in consultation with veterinary staff. Continual physiological monitoring and assessment (e.g. paw pinch reflex) will ensure an appropriate level of anaesthesia throughout the surgery. Good aseptic surgical techniques will minimise the risk of post-surgical infection. The risk of death from anaesthetic or surgical complications will be minimised by ensuring correct anaesthetic dosage (inhalation anaesthetics will be used wherever possible), maintaining fluid balance with sub-cutaneous injection of saline, and maintaining body temperature. General surgical complications will be minimised by applying good, properly trained surgical techniques and by appropriate action in the event of complications. During the acute phase following surgery, the animal is carefully monitored for any signs of deviation from the normal recovery process. Additionally, special attention is given during the first week after surgery to observe any changes in behaviour or appearance that may occur.

It is essential for our experiments that mice are in good health in order to be able to perform behavioural tasks, and we will carefully monitor all animals for any unexpected adverse effects.

Mice are expected only to experience controlled weight loss (85% of initial weight) during periods of food restriction, and this step is required to motivate the animals to perform behavioural tasks.

Throughout the food restriction period, animals are individually inspected, weighed and fed daily. Mice are carefully monitored for weight loss to ensure the weight remains above the specified limit. To prevent the animal's weight from dropping below 85%, we may maintain



the animal's weight at a higher level than 85%. We normally maintain food restriction throughout the duration of behavioural testing, given that we do not observe adverse effects.

Severe harms

In some animals, the use of stress-inducing procedures is needed to cause behavioural and neural phenotypes that allow us to study the effects of stress associated with anxiety disorders, which is our key scientific objective. Typically, mice exhibit a standard reaction to electric shocks involving jumping, brief freezing for a few seconds (i.e., remaining motionless except for essential breathing movements), and returning to their regular behaviour. While the intensity and quantity of shocks applied do not result in lasting physical harm to the animals, they are sufficient to create an aversive memory that can influence their later performance in behavioural tasks employed in this protocol.

For mice exposed to stress-inducing paradigms, overall clinical signs are not expected: we rather expect to observe specific alterations (e.g., freezing) in specific behavioural tasks.

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)?

Mice: Mild: 74%

Mice: Moderate: 23%

Mice: Severe: 3%

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

- Killed

A retrospective assessment of these predicted harms will be due by 25 January 2030

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 determine how sensory information is processed in the brain through the development of stress-induced anxiety. This goal requires the study of neural circuits in an intact behaving animal. Human studies allow us also to measure brain activity, but these studies do not allow us to assess brain activity in as much detail as in mice. We achieve much greater detail in mice by placing electrodes in the brain under anaesthesia and subsequently using them to measure the electrical activity of single brain cells during perception and behaviour. We can also genetically label brain cells so that we



can use microscopy to measure the activity of these cells through a small transparent glass window placed in the skull. This is necessary to understand which precise brain activity patterns cause specific behaviours.

Alternative experimentally valid approaches in silico (i.e., by means of computer modelling) or in-vitro (i.e., done outside a living organism) are currently unavailable.

However, where possible, to help interpret our data, we will take advantage of ex-vivo techniques (i.e., measurements done in or on tissue in an artificial environment outside the organism with the minimum alteration of natural conditions). For example, after completing experiments in-vivo (i.e. on living mice), we will characterise electrode tracks and the cell type of imaged cells ex-vivo.

We currently lack detailed data to create accurate models showing how neurons in various parts of the brain work together, how the circuit changes when stress-induced anxiety develops, and how different brain areas influence behavior. This project will generate data that will allow the development of statistical and computational models of sensory processing during stress-induced anxiety. We actively drive this work by collaborating and sharing our experimental data with computational neuroscience groups. Currently, no techniques are available that allow us to causally study neural circuits in humans with the required cellular resolution.

However, we closely interact with human neuroscience labs to seek translation of results between mice and humans (e.g. in this project, by testing hypotheses suggested by work in humans and primates, and by generating hypotheses that can be tested with more macroscopic methods in humans).

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

Computer simulations and tissue cultures, including brain organoids (i.e. self-organizing three- dimensional tissue derived from human stem cells).

Why were they not suitable?

Computer simulation cannot replace animal use because the modelling of brain activity during behaviour is not yet advanced enough to provide the required level of detail to answer our research questions. Furthermore, artificial computer simulations and tissue cultures (including brain organoids) cannot still form a translationally meaningful functional circuit that exhibit natural behaviour or engage in cognitive tasks.

A retrospective assessment of replacement will be due by 25 January 2030

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 used in this project will be minimised: I am aware of the PREPARE and ARRIVE guidelines (<https://proecopa.no/prepare>; <https://arriveguidelines.org>), and all our experiments are designed according to these guidelines.

Innovative techniques, such as optical imaging (i.e., technique that uses light to interrogate cellular and molecular function in the living body), chronic electrophysiological recordings (i.e., techniques that record the electric activity generated by neurons), and causal manipulations of the neural circuits (i.e., cell-specific activation/inactivation) will be used to obtain more information from the same animal.

We can reduce mice use by using simultaneous recordings from hundreds of cells and reversible causal manipulation in the same experimental session. The number of animals generated is dictated by the number required by our experiments to fulfil our scientific aims. Based on our preliminary data and existing literature, we estimate group sizes of about 10 mice.

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

During the experimental design phase, we performed a thorough analysis of previously performed experiments by systematic reviews, following the guidelines indicated by specific online tools (Radboud UMC (<https://www.radboudumc.nl/en/research/departments/anesthesiology/meta-research-team/tools>) and step-by-step guides (e.g., “A step-by-step guide to systematically identify all relevant animal studies”, Leenaars et al., 2021 Laboratory Animals).

We also considered SYRCLE’s RoB tool (SYstematic Review Centre for Laboratory animal Experimentation) and the online guidance offered on the NC3Rs website (<https://nc3rs.org.uk/3rs-resources/3rs-advice-project-licence-applicants>).

We carefully estimated the number of animals needed for each experiment based on our preliminary data. We used preliminary tests to quantify the variability of our observations and performed a power calculation to establish the number of mice required for each set of experiments. Based on our preliminary data, we estimate group sizes of about 10 mice.

We will use long-term neural measurement techniques to record responses of large numbers of cells in different brain areas, different time points, and different behavioural tasks within the same animal. This approach limits the number of animals needed and enables statistical within-animal comparisons (increasing statistical power). We will use the minimal number of animals per experiment, which gives sufficient statistical power to detect experimental effects and make scientifically valid conclusions based on power calculations. We use our previous data for these power calculations, where we used comparable approaches to estimate our effect sizes, indicating we need approximately 10 mice in each experimental condition.

Before each experiment, we use systematic literature searches (e.g., combined keyword searches in PubMed) to review relevant previous work to ensure our experiments are



necessary to answer our hypotheses.

Raw data, associated metadata, and analysis scripts will be stored on hard drives and backed up on Network-Attached Storage (NAS) servers to avoid data loss and enable data reuse (data will be shared where possible). We usually start experiments with a smaller set of animals, enabling us to check the quality of the data (comparing the data to previously collected data and ensuring all relevant information for analysis is being stored correctly). This may also enable us to sometimes reduce the number of experimental conditions (e.g. if the effects of two pharmacological dosages are comparable). We further optimise the statistical power for the selected number of animals in multiple ways. We maximise the yield from each animal, for example, by using volumetric scanning methods (i.e., the process of acquiring three-dimensional images of brain tissue) to increase the number of cells imaged simultaneously. We standardise experimental procedures to reduce measurement variability.

We use statistical testing that considers the variability unrelated to the experimental manipulations, for example, by using paired comparisons and multilevel analysis (Aarts et al. 2014, Nature Neuroscience). Where possible, we will use blinding (e.g. when comparing the genetic mouse model to littermates, genotyping results will be kept unknown to the experimenter during data acquisition, for example, to avoid any biases during behavioural testing). Where possible, we will randomly allocate animals to experimental groups. We will randomise experimental manipulations (e.g., the order of different visual stimulation trials during learning) to reduce bias. Detailed behavioural monitoring (e.g. eyes, running speed, licking) will allow control for variability in neural activity due to behavioural changes. Experiments are planned in accordance with the PREPARE guidelines, and results will be reported in accordance with the ARRIVE guideline (to maximise the information from the research and minimise unnecessary studies). To optimise our experimental design, we will take advantage of the NC3Rs EDA-Experimental design assist (<https://eda.nc3rs.org.uk/>).

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

In order to avoid the unnecessary use of animals for breeding, we will closely monitor our breeding colonies (and reduce the number of breeding pairs if possible) to avoid producing more animals than needed for experiments and colony maintenance.

We will initially perform additional pilot studies with 4 to 6 mice to quantify the variability of our data and optimise the number of animals required for each set of experiments. Where possible, to help reduce the overall number of animals used, animals implanted with recording electrodes and cranial windows for chronic imaging will be shared with other research groups that use similar techniques. We will take advantage of our internal 3Rs Enquiries list that supports tissue sharing.

A retrospective assessment of reduction will be due by 25 January 2030

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 mouse is currently a prominent model in sensory neuroscience and is best suited for the proposed experiments for the following reasons.

First, the anatomical and functional organisation of the mouse sensory system is relatively similar to humans and primates, including, for example, strong sensory feature selectivity in single neurons and organisation of sensory cortex.

Second, there are unique methods available in mice that allow detailed measurement and manipulation of brain circuits. This includes genetic calcium indicators (i.e., proteins engineered to observe neural activity) that allow for long-term measurement from large populations of cells and their projections; chronic electrophysiology with multichannel electrodes, that allow for neural recordings with extremely high temporal and spatial resolutions (these techniques allow us to record from as many neurons as possible in single trials, therefore decreasing the overall numbers of recording sessions on each animal, and, in general, the overall number of the animals used); transgenic mouse lines that express calcium indicators and allow for cell type-specific targeting; and mouse models of brain disorders.

Third, the size of the mouse cortex (i.e., the brain area on which our research is focused) is relatively small. This enables simultaneous measurement from multiple targets, which is not possible in larger mammals.

Finally, mice are capable of complex sensory and avoidance behaviours that mirror essential aspects of human and primate behaviour (e.g. stress-induced anxiety, visual detection and discrimination tasks). The combination of these factors makes it possible to use the mouse instead of higher mammals to address our experimental questions, while this is not currently feasible in lower animals.

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

We use mice because they are the lowest species that provide adequate physiological, anatomical and behavioural repertoires for the proposed research. The complexity of the processes described in this study (e.g., development of stress-induced anxiety) cannot be duplicated or modelled in simpler systems. Investigating the neural circuit mechanisms that underlie cognitive processes and memory consolidation necessitates the use of a living animal that can freely behave. Simplified experimental settings like tissue culture or artificial computer simulations cannot adequately explore the behavioural functions of the mammalian brain.



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

The mice in this project participate in long-term experiments in which they typically learn associations between sensory inputs such as specific visual objects and food rewards during behavioural tests. It is, therefore, necessary that animals are in excellent health conditions and not stressed. To reduce stress, animals are acclimatised to experiments by handling and giving food rewards. To refine the animals' lives, we provide, for example, cage enrichment (e.g. running wheels). Additionally, we house animals together where possible. We provide acclimatisation and handling to habituate mice to experiments and reduce stress. We also house our mice in a reversed light-dark cycle, which means that the mice are in their active phase during experiments and undisturbed during their inactive phase. In general, we first perform experiments in a small subset of mice (typically 2-3 mice) and refine experimental testing procedures if possible or necessary (e.g. adjust doses of pharmacological agents) before testing the other mice in that experimental condition.

We continuously seek possibilities to refine procedures further and improve animal welfare in consultation with animal technicians, NACWO, NVS and other colleagues.

The severity of surgical procedures will be minimised by applying pre- and post-operative analgesia in consultation with veterinary staff. Continual physiological monitoring and assessment (e.g. paw pinch reflex) will ensure an appropriate level of anaesthesia throughout the surgery. Good aseptic surgical techniques will minimise the risk of post-surgical infection. The risk of death from anaesthetic or surgical complications will be minimised by ensuring correct anaesthetic dosage (inhalation anaesthetics will be used wherever possible), maintaining fluid balance with sub-cutaneous injection of saline, and maintaining body temperature. General surgical complications will be minimised by applying good, properly trained surgical techniques and by appropriate action in the event of complications (e.g., stemming blood loss following accidental vessel damage). Immediately post-surgery, animals will be kept warm, generally cared for, and observed regularly until fully recovered.

Animals will then be observed at least once daily for the first 5 post-operative days.

During stress induction protocols, biomarkers and neural recordings will help us evaluate the effects of different interventions on anxiety-related behaviours, and determine the minimal sufficient stimulation required to satisfy the scientific objectives of this project. To optimise these protocols further, we will use our established optogenetics approaches ("optogenetics" is a biological technique to control the activity of neurons or other cell types with light), capitalising on the exceptional temporal and spatial control and modality specificity of our optogenetic toolbox. Animals are monitored closely, and if we observe any unexpected adverse effects, we will consult specially trained staff and vets. If the animal cannot be treated effectively, then it is humanely killed to avoid any potential suffering that may otherwise arise.

Pilot Study Plan for the refinement of the stress induction protocol

In our quest for more precise stress induction protocols, our pilot studies aim to determine the best parameters for electric shock sessions. We want to know the least shock intensity, the number per session, and the optimal repetitions to trigger specific physiological and behavioural responses. Our overall goal is to be able to get the stress repose required while ensuring that animals experience the lowest current intensity for the



fewest occasions.

We start by conducting sessions with minimal shock exposure, beginning with just five shocks. From there, we systematically vary the number of shocks within each session and overall session repetitions while closely watching the dose-response relationships. To fully understand the impact of shock parameters, we use various strategies.

The Dose-Response Curve method involves administering shocks with varying intensities, letting us see the resulting behavioural and neuronal effects. Plotting these curves, we gain insights into the nuanced relationship between shock intensity and the desired responses.

Another method is the Threshold Determination strategy, where we gradually escalate shock intensity until the first observable effect is noted. This helps us pinpoint the intensity just above the threshold, representing the minimal sufficient stimulation required.

To make sure our observations are specific, we incorporate control groups receiving non-active stimulation. This baseline comparison is vital for distinguishing effects attributable to stimulation from other potential factors.

Our evaluation criteria include a holistic assessment, considering both behavioural and neuronal measures. Biomarkers such as cortisol and corticosterone levels are used as indicators of the stress response. Our considerations go beyond just inducing stress; we strive to achieve this state while minimizing any adverse effects on the well-being of our animal subjects.

Continuous behavioural monitoring is a crucial aspect of our approach. We diligently observe animals during and post-shock sessions, looking out for signs of distress, adaptation, or habituation. Real-time adjustments to the protocol are made based on these behavioural observations, ensuring an adaptive and responsive methodology.

As we navigate through the pilot study, adaptive protocols come into play. Initial results guide modifications to the protocol, with a keen focus on optimizing shock parameters. We are committed to making necessary adjustments if the desired stress responses can be achieved with fewer shocks.

Ethical considerations form a foundational aspect of our pilot study. Prioritizing animal welfare, we implement measures such as acclimatization, gentle handling, and the provision of food rewards. Animals are closely monitored, and any unexpected adverse effects trigger consultation with specially trained staff and veterinarians. Our commitment to continuous refinement is embedded in the scientific decision-making process. Empirical data guides our journey, with the ultimate goal of achieving the minimum sufficient stimulation while upholding the welfare of our animal subjects. This iterative and adaptive approach ensures that our protocol aligns seamlessly with our scientific objectives.

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

My research adheres to and is guided by the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE), the Animal Research: Reporting In Vivo Experiments (ARRIVE) Guidelines, and the Laboratory Animal Science Association (LASA). My research is inspired by LASA's position on the



3Rs (https://www.lasa.co.uk/wp-content/uploads/2018/05/Position_3Rs.pdf), and I strictly follow LASA's guiding principles of aseptic surgery (<http://www.lasa.co.uk/wp-content/uploads/2017/04/Aseptic-surgery-final.pdf>). I actively strive to advance these principles as part of my ongoing refinement strategy, particularly regarding craniotomies (i.e., openings in the skull) and brain surgeries. I stay updated on best practices through direct updates from NC3Rs via their monthly newsletter (<https://nc3rs.org.uk/nc3rs-newsletters>). I am familiar with the best practice for head fixation described in "Refinements to rodent head fixation and fluid/food control for neuroscience" (2022), and I follow the recommendations proposed in this study. I follow the recommendations on head fixation surgery, motivation and reward, and refinement of the behavioural set-ups indicated in "Refining the use of head fixation and fluid control in rodents" - NC3Rs (<https://www.nc3rs.org.uk/3rs-resources/refining-use-head-fixation-and-fluid-control-rodents>).

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

I possess multiple sources of information that help us stay updated on advancements in the 3Rs and effectively incorporate them. I have a solid working rapport with the animal care staff in our animal facility, which aids in implementing progress in the 3Rs. I stay up to date with all the latest news from the NC3Rs via their monthly newsletter (<https://nc3rs.org.uk/nc3rs-newsletters>). I will stay informed about advances in the 3Rs by using internal 3Rs Search tools, and Norecopa (<https://norecopa.no/>), which represent two excellent sources for new 3Rs advancements. I receive substantial support from the establishment's staff, and the Named Information Officer provides me with regular critical updates, which I attentively review and share with all my lab members. I frequently organise workshops involving all group members to discuss changes in procedures related to project licenses, and I am an active participant in an international network of rodent users, engaging in regular meetings and sharing best practices.

A retrospective assessment of refinement will be due by 25 January 2030

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. Assessment of novel treatments and therapies against viral infections

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

Viral, Infection, Respiratory, Vaccine, Immunomodulation

Animal types	Life stages
Mice	adult, aged
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?

This project aims to develop novel therapies to treat viral infections.



A retrospective assessment of these aims will be due by 29 January 2030

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?

Viral infections continue to be a major health problem throughout both the developed and developing world. The huge potential for the development of new treatments and improved vaccines to treat, prevent and greatly reduce the incidence of life threatening and disabling infectious diseases and epidemics continues to drive research in this area, for which relevant and reliable animal models are required. Viral infections continue to be a major health problem throughout both the developed and developing world. The huge potential for the development of new treatments and improved vaccines to treat, prevent and greatly reduce the incidence of life threatening and disabling infectious diseases and epidemics continues to drive research in this area, for which relevant and reliable animal models are required.

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

The data generated under this project will provide information on lead compounds' efficacy:

- (1) New information on beneficial effects when compared to untreated/placebo treated controls
- (2) New information on beneficial effects when compared to a reference drug i.e., a drug known to have an effect on infection.

All data generated will be analysed prior to being recorded in our archives (electronic files and/or hard copies). All data from a given study will be compiled into a report comprising: (i) a short introduction including the aim(s) of the study, (ii) a materials and methods section including an outline of the experimental protocol, a summary of the experimental groups and a description of each readout, (iii) a results section including graphical representations of the analysed data, statistical analyses and interpretations, (iv) a conclusion section with a summary of all findings and (v) a raw data section. The study outcome, whether judged 'positive' (e.g., beneficial effect of the lead compound when compared to a reference drug) or 'negative' (e.g., no beneficial effect of the lead compound when compared to untreated or placebo treated and/or no greater beneficial



effect of the lead compound when compared to a reference drug and/or observed adverse events) will be reported.

The data generated will typically be sufficient for the study sponsor to decide the lead compound's future: (i) assessment of toxicology prior to being tested in the clinic, the next step in the drug discovery process leading to a novel therapy for an autoimmune or inflammatory disease, (ii) additional studies for further assessment of effectiveness of the test compound and its activity or (iii) rejection for lack of safety or efficacy.

Initial assessment in vivo may help to determine how well tolerated the compound is, whether any clinical signs are observed following administration. In addition, whether the compound is stable following administration and the duration and location within the body in order to determine the best route and frequency of administration to optimise effectiveness.

Our expertise allows the study sponsors to obtain advice on the most suitable model to test their lead compound. The interactions between us and the study sponsors at the study design stage and during the studies will allow preventing, anticipating or rapidly reacting to potential adverse events.

Some data may be used to generate scientific literature. These data are made available to the scientific community (i) during scientific conferences attended by us or our clients, (ii) upon request through our website or (iii) through targeted email campaigns and social media.

Some data may be used for statistical analysis in order to refine experimental designs and experimental methods and to reduce the number of animals used.

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

Work carried out under this license will test lead compounds or vaccines for their safety, effectiveness, stability and distribution and strength, compared to a known drug or vaccine (i.e., a clinically relevant drug which has been granted authorisation by the centre of the Medicines and Healthcare Products Regulatory Agency (MHRA)). By contributing to the development of new treatments and vaccines against viral infections, our project will benefit patients worldwide, providing targeted, effective treatment or effective vaccination reducing the incidence and spread of infection, and thus, reducing mortality. The recent COVID-19 pandemic highlights the benefit of providing safe and effective vaccines to the population.

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

The outputs of this work will help guide next steps. Where possible data will be shared at conferences, social media and in publications. The data will aid future studies, add to our experience to advise clients, and provide information to other scientists.



Species and numbers of animals expected to be used

- Mice: 7000
- Rats: 500

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.

Although many human viral infections do not affect rodents, we are still able to use them successfully in many models. Due to their size, ease of reproduction and handling, many aspects of the complexity of the interactions between multiple body systems that are critical to understanding these diseases and in defining treatments cannot. The use of robust in vivo models is therefore essential for the assessment of potential novel vaccines and therapies.

Adult animals are generally the most appropriate to use, however, occasionally we may need to use neonates, newborns, juveniles or aged animals to better assess the impact of a treatment prior to, or at time of disease onset, particularly where development of disease is spontaneous and may occur early or later in life. Further, the immune response can be affected by age, so use of aged animals in some models may also be required.

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

The following animal models will be used during the course of this licence:

- Influenza virus infection
- Herpesvirus infection
- Respiratory syncytial virus infection
- Intravenous infection
- Immunomodulation of viral infection
- Assessment of Immunization and modulation of immune responses.

Mice will be preferred for most studies, but rats will be used when the lead compound is not suitable for use in mice, the rat provides a more clinically relevant model and endpoints, or a larger volume of sampling for ex vivo analysis is required.

Infection will be established following administration of virus by a variety of routes depending on the model of choice e.g., into the lungs by inhalation or direct into the trachea for respiratory viruses. For the models listed, we have validated data for frequently



used viral strains. Where other strains of virus that have not previously been established at this Establishment, in the strain of animal required, a small study will be carried out in which groups of 3-5 animals will receive virus in the range expected to establish infection. The lowest number of animals per dose (e.g., 3 per group), will be used to efficiently assess the correct dose required for future studies. However, where virulence and variability are unpredictable, higher group sizes may be used to ensure experiments are informative and reproducible. Animals will be monitored as described in the protocol and subsequent experiments will use the lowest dose at which reliable infection is achieved, thus minimising the level of suffering, and avoiding the use of doses which cause animals to reach a moribund state.

Therapies may be given prior to or following infection. For example, the testing of vaccines required their administration prior to the establishment of infection, whereas the administration of novel anti-viral compounds would normally be post-infection in order to mimic likely clinical protocols. The most appropriate route will be selected based on an understanding of the stability and durability of the therapy being given. Analysis of excretions at various time points following administration of compounds, may also be carried out in order to determine the optimum dosing regimen.

In some cases, evaluation of mechanism of action may require inhibition of some aspects of the immune response to prevent additional damage to organs. Alteration of such responses may reduce or exacerbate disease related symptoms, requiring extra checks to be put in place to minimise any unexpected suffering.

In order to compare immune responses and amount of virus present in blood and organs at the point at which untreated/placebo treated control animals reach peak infection, additional animals may be included (satellite animals) for termination at this point. This is usually involves small groups of 3 animals. Remaining animals continue for assessment of disease course.

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

Most animals will undergo a viral challenge, which will lead to observations of clinical signs, such as change to coat condition, changes to posture, reduction in activity, discharge to the eyes and altered breathing patterns, in the absence of treatment. In efficacy studies animals will also be given a treatment, by a suitable route prior to or after infection.

Some animals may be used to test a vaccination, which involves one or two injections prior to any challenge. To assess the efficacy of the vaccine blood samples may be taken during this time. If the blood samples do not show the expected, then these animals may not be challenged, so would not have any further adverse effects.

If a new viral strain is being tested a dose response will be required, which would include a greater amount of the virus being administered to a small selection of animals than



anticipated to be necessary. In these circumstances animals will be monitored more regularly to ensure that the animal does not go beyond the humane endpoints.

Typically, following viral infection, untreated animals develop signs of clinical disease and weight loss over 7-10 days. Where treatment reduces clinical signs, animals may be maintained beyond 10 days to ensure longevity of treatment or immune response to prevent re-infection. Where vaccines are to be assessed, these may be administered on a single or multiple occasions at 1-4 week intervals prior to infection, thus increasing duration of such studies by up to 12 weeks.

For vaccination studies, antigens may be given in an appropriate formulation to help induce an effective response (adjuvant e.g., Freund's complete) which themselves, may cause some additional adverse effects such as reaction at the injection site. Use of some adjuvants may therefore be limited.

Animals may be imaged under anaesthesia to track either the virus or administered compounds. In this case an imaging substrate may also be required to be injected prior to imaging. If substrate is required, no more than 2 or 3 imaging sessions will be performed in 24 hours. Otherwise, up to 5 imaging sessions may be performed within 24 hours. Typically, this would be daily imaging only.

Animals may be confined, singly, or in small groups, for a period not exceeding 2 hours (AA) before, during, and/or after dose administration for collection of excreta.

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)

As most protocols involve the development of clinical signs associated with infection, the majority of mice and rats, approximately 90%, are expected to experience moderate severity. A few naïve animals, <10%, may be included to provide baseline levels of disease, cytokines, cell proportions or numbers, for comparison with treated and untreated controls. Equally, a proportion of animals may not progress to challenge due to lack of responsiveness to treatment and will therefore experience no more than mild clinical signs (<10%)

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

- Killed

A retrospective assessment of these predicted harms will be due by 29 January 2030

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 interaction between host and pathogen is an important driver of expression of different virulence factors. While some individual aspects of these processes can be modelled in vitro, the complexity of the interactions between multiple body systems that is critical to understanding these diseases and in defining treatments cannot. The use of robust in vivo models is therefore essential for the assessment of potential novel vaccines and therapies.

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

Clients will often provide data from assays already performed that indicate the potential of a compound to reduce the onset of viral infections. In the absence of such data, in vitro assessment may be required before progressing to an animal model. Potential antiviral compounds are initially assessed for efficacy in cell based systems, typically assessment in viral assays and determination of the concentration of drug that kills 50% of the virus or prevents virus from infecting 50% of cells (effective concentration (EC50)). Where appropriate susceptible cell cultures are available, assessment of changes in the signals secreted by the cells may be carried out, including, if possible, use of human derived cells. We have a number of in vitro cell based assays including use of transwells and 2D models. We are also developing the use of organoids and tissue on a chip models for assessment of test compounds prior to testing in animals. In addition, the wax moth larvae, *Galleria melanogaster* model, may also be used as an intermediate between in vitro assays and rodent models, as this has a basic innate response, enabling some aspects of cellular interactions to be assessed in context of the therapeutic of interest.

Why were they not suitable?

Alternatives to animal models are a great addition in the assessment of test compounds and can help to confirm mode of action. Most test compounds have been assessed in vitro by the sponsor or by our Immunology Team, however due to the additional complexity of the human body and interactions of immune cells and pathways, it is not possible to replicate this in vitro, and thus in vivo studies are unfortunately still required to fully understand and show efficacy.

A retrospective assessment of replacement will be due by 29 January 2030

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 is estimated from previous animal returns.

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

Use of in vitro assays, including potential 2 and 3-dimensional set-ups, will minimise the number of animals required for initial assessment of potential novel compounds. In addition, utilising the National centre for implementation of replacement, reduction and refinement of animal use in research (NC3R's) guidelines and the experimental design assistant, can help to balance group sizes. Numbers of naïve, untreated or vehicle treated animals will be kept to a minimum while still allowing meaningful data to be assessed for statistical significance. Objective of the study (e.g., a dose finding or efficacy) as well as the incidence of disease and variability of the model will impact group sizes. Assessment of previous data or review of the literature will also help guide the group sizes as well as the number of control groups required. While we work to Good Laboratory Practice (GLP)-like standards, however, we do not have to comply to additional regulatory guidelines.

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

Understanding from the literature and breeding establishments the expected incidence of disease and variability of the model will help ensure group numbers are appropriate. Assessment of previous data or review of the literature will also help guide the group sizes as well as ensuring appropriate control groups included.

A retrospective assessment of reduction will be due by 29 January 2030

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.

Appropriate models are assessed by review of the literature to ensure the most up to date refined model is used. Reducing where possible the number of administrations required, minimising administration volumes and sampling frequency and volume, while still ensuring reproducible and informative data. We will use the shortest model that will provide us with suitable disease profile and incidence of disease for assessment of treatments.

All models described here are well characterized and reproducible for assessment of novel therapies. Respiratory models of disease such as influenza provide an effective model for assessment of viral load and antiviral drugs, as well as novel vaccination methods for induction or inhibition of immune responses. Other respiratory models such as RSV will be used for targeting specific pathways involved in RSV infections. Herpes virus can cause disease at different mucosal surfaces including the eye and genital tract and becomes latent in the nervous system, where it can be reactivated leading to recurrent infections. Such infections require novel approaches to control disease incidence and severity. The choice of model will be dependent on the drug target or vaccine strategy.

Use of specific strains or GA mice can provide a more relevant model of disease for assessment of potential therapies such models will therefore be used where appropriate.

Where a temperature drop may occur subcutaneously implanted transponders will be used to allow close monitoring of the animal's condition. Where feasible in-life imaging may be performed allowing information to be gathered at various timepoints, without increasing the number of animals required. Furthermore, viral swabbing can indicate proliferation of a virus which can allow for closer monitoring of these animals to ensure there is no unnecessary suffering. In addition, this may reduce the need for satellite groups for assessment of viral load during the course of the disease.

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

Most of our studies will be done in mice, which are the lowest species that develop these diseases in the same way as humans. Rats are occasionally used when the disease cannot be modelled in mice, if the test compound does not work appropriately in mice, or if a larger animal is needed for sampling requirements.



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

Animal suffering will be limited by ensuring that the models used cause the least amount of harm to the animals. The mildest disease inducing agent or 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 objects 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.

Animals are provided with a bowl of mashed food on the cage floor if moving may be uncomfortable. When substances need to be administered, we will give the smallest volume possible and administer it in the way that causes the least distress.

We employ the use of temperature transponders, IVIS imaging and viral swabbing to track the progress of the disease and provide guidance on the animals' condition, so allowing suffering to be curtailed.

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

We follow the recommended Best Practice guidelines of Turner et al. 2011 and Diehl 2001. We keep up to date by receiving regular updates from the Home Office (HO), Laboratory Animal Science Association LASA, IAT and the National centre for replacement, refinement and reduction (NC3Rs) as well as following the literature, internal forums and advice from our dedicated veterinary surgeon.

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

We keep up to date with NC3Rs guidelines by online updates as well as attending Institute of Animal Technology (IAT) and Laboratory Animal Science Association (LASA) conferences, Home Office Liaison, Training and Information Forum (HOLTIF) and workshops.

We also check the literature and consult with named personnel where required, to ensure the models we are using are the most appropriate and include additional validation of models where improvement can be made.



A retrospective assessment of refinement will be due by 29 January 2030

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. Developing treatments for dangerous pathogens

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

Infectious disease, Pathogen, Vaccine, Therapeutic

Animal types	Life stages
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

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 work is to assess if medicines (such as antibiotics and vaccines) can effectively prevent or treat disease caused by dangerous pathogens, using a non-human primate model.

A retrospective assessment of these aims will be due by 6 February 2030

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?

Infectious diseases account for approximately a quarter of deaths globally and approximately 7 % of deaths in the UK. Such diseases are a significant health and economic burden. There is an enduring risk to the UK from highly infectious pathogens that can cause lethal disease and that do not routinely circulate in the population. With such pathogens, there is no natural immunity to the disease and often there are no medicines that can treat or prevent disease. Therefore, the consequences of an outbreak of disease can be devastating, with a large number of people becoming sick and often dying. The work undertaken in this licence will assess existing or novel medicines for their potential to prevent or treat disease.

The data may be used to support and / or as pre-clinical studies for licensure of medicinal products and ultimately save lives.

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

The main output of this work in the short term will be new knowledge about the success (or otherwise) of the treatments assessed in this project that are expected to protect against disease caused by dangerous pathogens. This knowledge could help improve treatment for people exposed to dangerous pathogens.

In addition, knowledge generated during this work will lead to a greater scientific understanding of the disease process for any new infectious disease models developed. This will help identify potential new medical intervention opportunities, diagnostic approaches and medicines.

Work will be written up for publication in peer reviewed journals and aspects of the work will be presented both nationally and internationally at scientific conferences.

In the longer term, the aspiration is for knowledge generated in this work to form part of a data package submission for licensure of novel products or indications and/or inform policy decisions.

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

In the short-term, outputs from this work will help inform new medical interventions and diagnostic approaches which will protect people in the UK, as well as providing information for medics in regions where the disease more regularly occurs.

In the longer term, this work and the knowledge gained can help develop future strategies and technologies that can shorten development of medical approaches in e.g. epidemic and pandemic outbreaks, to help civilian populations worldwide.

Ultimately the outputs from this, and likely future follow-on work, have the potential to save



lives (both at home and in regions where the disease regularly occurs).

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

Work will be written for publication in peer-reviewed journals and/or for conference presentations.

Data will be shared at international meetings, through regular attendance at the appropriate Forums e.g. NC3R's primate Meeting, International Infectious Disease forums, customer data sharing workshops and international meetings.

Species and numbers of animals expected to be used.

- Marmosets: 280

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.

This work involves adult non-human primates. Non-human primates are biologically very similar to humans, with their fundamental biological features ensuring that their immune cells react to disease in the same way as humans and processing of drugs in the body uses the same mechanisms as humans. As such, they allow us to understand and replicate the human disease with accuracy and discover what drugs and vaccines can protect humans from the disease.

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

Most animals will be used to assess how well vaccines or drugs protect against or treat disease. For these studies, the general experimental approach will involve: Animals will have a device to monitor body temperature implanted in the abdominal cavity using surgery. This device allows continual monitoring of temperature and the implementation of humane endpoints at later stages of the experiment.

On a single occasion thereafter, animals will be exposed to pathogen. This will usually be delivered as an aerosol whilst the animal is anaesthetised, but sometimes the pathogen will be given to the animals by injection (e.g. sub-cutaneous) or by the oral route.

Before or after they are exposed to the pathogen depending on the nature of the medicine, animals will be given a vaccine or drug (or a placebo) to discover whether this prevents or treats disease. A vaccine may be given up to 5 times by injection using standard routes (e.g. intra-muscular, sub-cutaneous). The drugs may be given up to three times a day for up to 21 days, usually with the animal willingly taking it by mouth from a syringe. Some animals may breathe in the drug whilst anaesthetised or receive it by injection.

Blood and other samples (e.g. throat and nose swabs) will be collected on at least one occasion before challenge and up to 7 times after exposure to pathogen. The animal will be anaesthetised during the collection of these samples.



Some animals will be used in studies aiming to understand how vaccines or drugs are processed in the animal (e.g. the immune response to a vaccine or how quickly the drug is expelled or degraded) to ensure the treatment regimes used are similar to those used in humans. For these studies, the general experimental approach will involve:

Animals will be given a vaccine or drug (or a non-functional control substance) to determine the response of the animal to the treatment. A vaccine may be given up to 5 times by injection using standard routes (e.g. intra-muscular, sub-cutaneous). The drugs may be given three times a day for up to 21 days, usually with the animal willingly taking it by mouth from a syringe. Some animals may breathe in the drug whilst anaesthetised or receive it by injection.

Blood will be collected up to 8 times to allow drug levels to be quantified.

A small number of studies will look at the how a pathogen causes disease in an animal in the absence of and therapeutic intervention. The aim of this is to to develop models that can be used to study treatment and ensure the models are suitable. For these studies, the general experimental approach will involve:

Animals will have a device to monitor body temperature implanted in the abdominal cavity using surgery. This device allows continual monitoring of temperature and the implementation of humane endpoints at later stages of the experiment.

On a single occasion thereafter, animals will be exposed to pathogen. This will usually be delivered as an aerosol whilst the animal is anaesthetised, but sometimes the pathogen will be given to the animals by injection (e.g. sub-cutaneous) or by the oral route.

Blood and other samples (e.g. throat and nose swabs) will be collected on at least one occasion before challenge and up to 7 times after challenge. The animal will be anaesthetised during the collection of these samples.

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

The most significant impact that most animals may experience is that they will feel unwell from the disease caused by the pathogen. Animals are likely to get an increase in temperature for between 2 and 7 days as their body fights the infection. During this time they may appear tired and subdued, not move around as much as normal and not groom as much as usual. They may breathe more quickly than normal. In a small number of animals these signs will increase in severity, which would ultimately lead to death if a humane endpoint were not applied. A small number of animals may also become confused or disorientated.

The next biggest impact would be due to the surgery to implant the temperature monitoring device. Surgery wounds typically take 3-4 weeks to fully heal due to grooming of socially housed animals. However, this short-term impact on the animal has a long term benefit as the temperature device allows us to constantly monitor animals and forms part of any decision to kill animals when they have reached pre-defined endpoints, overall reducing any potential suffering.

There is likely to be bruising at the site of blood sampling. This would be expected to resolve within a week or two.



Vaccines and medicines given to animals are not expected to cause harm to the 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)?

Sub-threshold severity 2%

Mild severity 19%

Moderate severity 67%

Severe severity 12%

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 6 February 2030

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 interactions between a pathogen, the host it infects and any treatments given to the host are extremely complex. This is especially true because the primary interface includes the host immune system, an incredibly interconnected and ever changing network of host responses involving virtually every cell type in the body. Although some isolated aspects of these host-pathogen, host-treatment and pathogen-treatment interactions can be recapitulated outside of animals in the laboratory or in computer simulations, these processes are so complex that currently no non-animal system is able to replicate this.

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

The work undertaken on this Project licence is part of a larger research programme with the aim to identify medicines for a number of pathogens. Research and identification of these medicines are at various stages of maturity within the research programme. Research on each pathogen starts with a literature search to identify appropriate models (e.g. *in vitro*, *ex vivo*, *in silico*, non-vertebrate, small animal models and NHP models). The research programme has enduring activity in all these areas. Work will only be undertaken on this licence for the most advanced candidates that have been proven to be effective in the other systems / models.



Why were they not suitable?

Cellular infection assays and invertebrate animal models are simplistic models, which to date, are unable to replicate all aspects of the host response to pathogens, and the protective response elicited by the drug/vaccine. These are useful tools to understand and interpret aspects of the response, but are not able to accurately predict the outcome in humans.

A retrospective assessment of replacement will be due by 6 February 2030

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?

These numbers are based on the projected funding over the next 5 years, taking into account the number of studies that could be performed based on facility space, animal availability and demand. The estimation is based on performing:

Development of two new models of disease, one using 32 animals and one using 44 animals for the whole model development process (76 animals).

Four studies of how the animals respond to administered medicinal products, each using 18 animals for the whole process (72 animals).

One study assessing how vaccines stimulate the immune system, using 12 animals (12 animals).

Evaluation of four therapeutics, each using 30 animals for the whole process (120 animals)

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

In-house statisticians are involved with the experimental design and data analysis to ensure the best balance is achieved between keeping animal numbers as low as possible whilst having the highest likelihood of achieving the scientific aims of the study. For studies involving larger numbers of animals, studies are performed in a stratified manner with interim statistical analysis determining whether it is necessary to continue with the next phase.

Where possible, control animals will be shared between groups and the number of control animals will be reduced by using historical control data.



The maximum amount of information will be obtained from each animal and samples will be stored for future exploitation and for use in other programmes e.g. diagnostic programmes.

Animals that have not been infected with a pathogen may be re-used a maximum of 2 times (a total of 3 uses), where appropriate.

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

This work forms part of an integrated research programme, where data is generated *in vitro* and / or in non-animal alternatives prior to assessment in other animals species before moving into non-human primate studies.

In the case of repurposed drugs, available data from human populations (e.g. safety, pharmacokinetic information etc.) will be used to inform studies. Outputs from these studies will inform the experimental design and the appropriate number of animals required.

Pilot studies will be performed to determine the magnitude of the effect (protection or disease readout) and used to optimise the number of animals required for robust data. For studies involving larger numbers of animals, studies are performed with interim statistical analysis determining whether it is necessary to continue with the next phase.

Modelling of how drug levels within an animal change over time will be performed using data from pilot studies to model a variety of scenarios without the need to establish empirical data in all cases.

The maximum amount of information will be obtained from each animal and samples will be stored for future exploitation and for use in other programmes e.g. diagnostic programmes.

A retrospective assessment of reduction will be due by 6 February 2030

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 majority of animals will be infected with pathogen that will go on to cause serious and possibly lethal disease. Most of these animals will also receive treatment of some form (e.g. antibiotics, vaccine) that is expected to prevent or treat disease to some extent. Animals will have blood and other samples (e.g. nasal swabs) taken for analysis at various times throughout each study.

Animals in these studies will have a device surgically implanted prior to the study to allow remote monitoring of important physiological parameters, particularly body temperature. Implantation surgery is a significant trauma for the animal to undergo, but this allows the course of disease to be closely monitored and for humane endpoints to be implemented as soon as possible. This limits the time the animal spends experiencing disease and is thus considered the more refined approach despite the trauma caused by the surgery. Animals will receive appropriate drugs during and after surgery to relieve pain and will only be released for further use when surgical wounds have been assessed as fully healed by a veterinarian.

To induce disease, animals will be infected with pathogen by mouth, subcutaneous injection or inhalation of aerosols. Anaesthesia will be used during aerosol infections to minimise distress caused during restraint for exposure.

Animals will receive treatment using methods similar to those used in humans e.g. by mouth, sub-cutaneous injection, intra-venous injection, intra-muscular injection, inhalation etc. Where possible, drugs will be administered by the oral route in a palatable formulation that the animals will take voluntarily from a syringe while in their home cage. Delivery of drugs via intra-venous injection and inhalation will take place under anaesthesia to minimise distress.

Blood and other samples will be taken under anaesthesia (where appropriate) to minimise distress.

Some animals will not be infected with pathogen, but will instead only receive treatment. This aims to understand the processing of the drug / vaccine in the animal to allow correct (human-like) doses to be chosen for use in the treatment studies.

Animals in these studies do not need to undergo surgical implantation of a telemetry device.

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

Non-human primates will only be used in late development studies for advanced products, following initial work in vitro and/or in less sentient animals. These animals are required as a final predictor of the efficacy of compounds in humans and so require species that have a highly evolved and relevant immunological and physiological response to the disease and compound.

We can't use embryos or very young animals as their immune system is immature and doesn't respond to pathogen in the way mature animals do.

The time to onset of disease and the effect of the medicines occurs over many days and weeks and it would not be possible to do this in terminally anaesthetised animals.

How will you refine the procedures you're using to minimise the welfare costs



(harms) for the animals?

Infected animals will be observed frequently and monitored using remote telemetry (CCTV, temperature implants). The frequency of welfare checks increases following the onset of fever, presence of clinical signs or any other observations that may indicate an advancement in disease. Animals approaching the humane endpoint will be constantly monitored, typically by CCTV supported by cage-side checks.

For animals undergoing any surgical procedure, appropriate pain relief will be given pre- and post- surgery. Animals will be monitored as they recover from anaesthesia and will be regularly checked post-surgery to ensure appropriate level of analgesia and monitor the progression of wound recovery. Sub-cuticular stitching will be used wherever possible to minimise any discomfort due to stitching.

Where appropriate, animals will be familiarised to the procedure being performed (e.g. handling, environment, personnel). Animals will be trained to receive liquids orally and only suitably trained animals are used in studies where oral delivery is required. Animals will be trained to willingly sit on a bucket for weighing purposes.

Animals will be habituated to personnel working in the different facilities prior to moving, and materials (e.g. buckets, scent marked perches etc) will be moved with the animal.

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

Key references include:

"A Good Practice Guide to the administration of substances and removal of blood, including routes and volumes."

"Opportunities for refinement of non-human primate vaccine studies".

The NC3Rs website will be monitored for new guides and the guidance regarding blood sampling will be followed.

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

Members of the team regularly attend the NC3R's Primate Meeting on an annual basis to present and share data with others in this field. The NC3Rs newsletter is distributed to all licence holders at this establishment. Updates on advances in the 3Rs are regularly distributed by our Named Information Officer. The licence holder is actively engaged in our establishment Animal Welfare and Ethical Review Body

Several of the team are members of the Institute of Animal Technology, and attend various fora where best practice guidelines are discussed and shared.

We have set up a network of primate users with similar researchers in the UK and we have regular formal and informal discussions, share best practices and lessons learnt as well as scientific data. We are part of many international forums with primate users and we have regular formal and informal discussions, share best practices and lessons learnt as well as scientific data.

A retrospective assessment of refinement will be due by 6 February 2030



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. Inflammation and cardiometabolic 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

Inflammation, Atherosclerosis, Obesity, Heart disease

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?

This project aims to examine the link between inflammation and heart disease. We want to investigate the mechanisms of disease, the molecules and pathways involved in the pathology and understand the complex interactions involved.

A retrospective assessment of these aims will be due by 6 February 2030

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?

Cardiovascular disease is the biggest cause of death Worldwide and is a massive social and economic burden on society. Current therapies focus on alleviating risk factors for the build-up of fat in arteries (atherosclerosis) such as high-cholesterol and high blood pressure. However despite novel therapies, 70% of events such as heart attacks cannot be prevented.

Work over the past 35 years has proven that inflammation is integral to atherosclerosis. A recent clinical trial has shown that reducing inflammation is beneficial in patients with cardiovascular disease. However, targeting the molecule assessed in the trial (IL-1 β) increases the risk of infection. Therefore, better therapies that more specifically target inflammation in atherosclerosis and other cardiometabolic diseases such as diabetes and obesity are needed. Obesity is an increasing problem that causes chronic inflammation which also contributes to the development of heart disease.

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

- Identification of molecules that promote/inhibit inflammation in cardiometabolic diseases such as atherosclerosis
- Increased understanding of the mechanisms/pathways and cell types involved in the development of cardiometabolic diseases
- Publications in peer reviewed journals related to our findings
- Identification of new targets for therapeutic treatment of patients

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

In the short- to medium-term, our outputs will benefit the scientific community both those working in similar fields of interest and those working in fields with similar features e.g. inflammation in cancer or neurodegeneration.

In the longer-term, the ability to identify points of intervention for therapeutic purposes would particularly benefit the pharmaceutical industry and patients.

Therapies to treat these conditions would be enormously important. 70% of events such as strokes and heart attacks and aneurysms cannot be prevented.

Cardiovascular disease is a huge social and economic burden on society. Biologicals/pharmaceutical agents or treatment strategies (e.g. prevention) could well be extracted from this work. Increased scientific knowledge could lead to reduced mortality, increased lifespan, improved quality of life and a reduced burden on healthcare systems.

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

We will endeavour to publish in open-access journals. Results will be presented at scientific meetings within the Establishment, Nationally and Worldwide. Data, animals and tissues will be made available to other researchers upon request and we will collaborate



with other researchers where possible.

Species and numbers of animals expected to be used

- Mice: 22,250

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.

Our project utilizes both wild type and genetically modified mice. We use mice as they are the best representation of the human as they share over 90% of genes.

Mice are easily genetically manipulated to create models of cardiovascular diseases and other relevant diseases such as obesity and diabetes. There is also a wealth of information and substances that have been designed and developed to be used with the mouse than in any other laboratory species.

Our experiments will use juvenile to adult animals as cardiometabolic diseases are chronic disorders that can occur over decades of an individual's life.

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

Genetically altered and germ-free mice will be bred using conventional methods. The mutations in our mice are not expected to cause any welfare concerns, however when they are of a germ-free health status, they may be more prone to gastrointestinal issues. Some mice will need to have the gene of interest activated by the administration of a substance which is typically Tamoxifen.

Some animals will be fed altered diets, such as high fat diet, to enhance or accelerate disease progression.

Some animals will receive substances that will modify the disease, cause a response by the immune system or be of a therapeutic nature. They may also receive substances that enable cells to be tracked or labelled to analysis their activity. The routes used are not expected to cause any harm that is more than transient and mild.

Some mice will be exposed to irradiation. This will deplete animals bone marrow and then they will be given bone marrow from another mouse to replace the depleted bone marrow. This process is like bone marrow transfusion in humans which is used to treat blood and immune systems diseases which affect the bone marrow.

Some mice will undergo surgery to place a collar on the carotid artery to injure the vessel. This is performed under general anaesthesia and the animal is given pain relief as a human would receive after surgery.

Some mice will have a small drug delivery device called an osmotic minipump fitted. This can be for one of two reasons. This is to remove the need to constantly inject a substance for an extended period or to administer a substance known as angiotensin II, which causes aneurysm formation. Typically, animals are humanely killed four weeks after the



administration of angiotensin II.

Some mice will have arthritis induced by the administration of either collagen or antigen. Around 50% these animals will also undergo surgery to cause a heart injury as well. These animals will be on study for around eight weeks.

Some animals will have inflammation induced by either an injection into the abdominal cavity or into an air pouch (created by injecting air under the skin on the animal's back).

Some animals will undergo blood sampling. This may be to analyse the effect of substances we have given or in some cases to monitor blood glucose levels. In some cases, studies can last as long as 20 weeks, but most are of a shorter duration. At the end of the experiment all animals will be humanely killed and tissue harvested post mortem for further analyses in the laboratory.

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

Exposure to irradiation may cause weight loss of up to 20% approximately 7 – 10 days post exposure. They may also exhibit a hunched posture, but all clinical signs should be resolved after 14 days.

Surgery to induce vascular injury should not cause an issue per se, however the injury to major blood vessels to the heart will increase the risk of the animal developing chronic heart disease which could lead to sudden death.

Animals that receive angiotensin II may experience an aneurysm and as a result of the rapid loss of blood may die.

Arthritic mice will have pain and swelling in one or more joints resulting in reduced mobility although they will be able to feed normally and move around.

Mice in which inflammation is induced in a cavity e.g. peritoneum/air pouch may experience mild discomfort/tenderness but general movement and feeding would not be affected.

Mice fed a modified diet may experience weight gain, soft stools and dermatitis, especially if a diet high in fat is 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 animal type)?

Sub-threshold: 40%

Mild: 20%

Moderate: 35%

Severe: 5%



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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 6 February 2030

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 animals models of disease to understand the cell types and molecules involved in the development and progression of pathology as cardiometabolic diseases are chronic disorders that can occur over decades of an individual's life. Inflammatory processes, especially in cardiometabolic diseases, are very complex and it involves both local tissue sites e.g. arteries and systemic sites such as lymphoid tissues. The diseases we study involve many different cell types communicating and interacting with one another. These scenarios cannot be replicated in vitro as they are too complicated.

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

Studies on human samples form a core part of our work and lead our experimental design. We obtain samples removed during heart surgery and we use the cells in these tissues to examine the molecules and cell types that are present. We can treat these cells with drugs and other substances to see if they affect e.g. the production of inflammatory mediators before we examine them in mouse models.

We also use blood cells from patients and healthy volunteers and cell lines to examine our aims and to test substances prior to performing in vivo experiments.

We regularly perform literature searches and attend seminars to keep abreast of any new techniques that may offer alternatives to procedures in animals.

Why were they not suitable?

Whilst they provide valuable information, studies on human samples do not permit retrieval of information on the development of the disease itself, or the history of any disease - it is a snapshot of disease at a late stage. Inflammation in chronic diseases is very complicated and can affect the whole body, not just the site of disease.

Animal models are also necessary because no in vitro techniques emulate the flow characteristics of blood vessels susceptible to atherosclerosis.

A retrospective assessment of replacement will be due by 6 February 2030



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 work will continue the work performed on previous project licences. Using the typical variation we have observed previously in our models, we have used statistical tests to calculate the minimum number of animals to be used whilst ensuring that the results are statistically significant. Sample sizes for our experiments are estimated from past experiments. Calculations typically show that we need group sizes of 8-10 (depending on the type of procedure) to achieve the quality of results we need.

We've used our annual return of procedures data to estimate the number of animals that we will need to use for breeding.

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

After consideration of in vitro models, any animal experiments required will be subject to rigorous experimental design. To ensure that the minimum number of animals will be used in this project, we will minimise experimental variation e.g. animals of the same sex, similar age, weight, fed on the same diet, provided with the same bedding. We will remove potential bias e.g. by blinding and by randomly assigning animals to experimental groups. We will use tools such as the NC3Rs Experimental Design App to assist with this. Whilst we generally use one sex of animal in each project to minimise variation, the other sex will often be used in a separate project.

We will endeavour that all experiments will follow the PREPARE guidelines and comply with the ARRIVE guidelines.

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

Breeding colonies will be managed in line with the best practice guidelines. Particular attention will be paid to genetic stability and good breeding performance. Data from breeding animals are readily available from the in-house database and will be used to make decisions on future breeding stock and to assist in maintaining a suitable colony size to ensure only those animals needed for experiments are produced. Cryopreservation of colonies not required in the short term will be considered.

After being humanely killed, we routinely collect as many tissues per mouse as possible. If not used immediately, these are shared with other researchers or stored long-term. For



each mouse we maximise the amount of information we can obtain and in some cases, one tissue is used for several experimental outputs. We have pioneered high-dimensional multi-parameter tests allowing up to 40 markers to be detected on each cell, minimising the number of experiments that need to be performed.

Where possible littermate controls will be used to further increase the sensitivity of our read-outs, untreated control groups may also be shared between treatment groups. In addition, untreated control groups will be used as a source of tissues for in vitro analysis.

A retrospective assessment of reduction will be due by 6 February 2030

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.

Our models of cardiovascular disease have been chosen based on their ability to generate the amount of disease required for our scientific outputs in the minimal time and with the lowest harm possible and we have extensive experience in performing them.

We will utilise two models of cardiovascular disease in our studies:

Surgical models – we perform a procedure to place a collar around the carotid artery which alters the blood flow or we surgically place the collar after we have performed surgery to completely block the artery. Both methods induce heart disease which replicates human disease but cause the animal minimal distress.

Diet induced model – we feed genetically susceptible animals a modified diet which results in the build-up of cholesterol in the arteries leading to atherosclerosis. This method is the most refined as the animal is not subjected to any procedures likely to cause stress but we can still achieve our scientific aims.

Aneurysms will be induced by inserting a small drug delivery device, known as an osmotic minipump that will deliver a substance called Angiotensin II. This causes weakening of the vessel wall. This is the gold standard method and most refined method as it does not need any major surgical intervention on the aorta. However as in the human patient some animals may suffer an aneurysm, resulting in sudden death prior to the end of the experiment.

For our arthritis model we will prioritise the antigen induced model as it is the least severe of our options and only gives rise to mild lameness rather than the more aggressive



collagen induced model which is more reflective of the human disease model.

We will utilise two models of inflammation which are both well used within the field.

The creation of an air pouch on the back of the animal and subsequent injection of an inflammatory agent is the least harmful of our models as it recruits the immune cells into the air pouch meaning there is no systemic inflammation. The other model involves an injection of an inflammatory agent into the peritoneal cavity which may lead to a more harmful systemic response for the animal. This peritonitis model creates a more systemic inflammation and involves a wider range of inflammatory cells and thus better mimics some inflammatory processes.

We will use imaging to track the disease progression. The methods we use may require the administration of fluorescent dyes to enable us to see the organ/injury of interest, but these dyes are non-toxic. Mice are anaesthetised during imaging, but it is not expected to cause any harm. This enables us to gain more data from each animal rather than the need to humanely kill animals at different time points.

We use substances such as tamoxifen and diphtheria toxin to induce genetic responses in some of our modified lines. This method means that the mutation is only activated in the relevant cells when required rather than breed the mice with harmful mutations.

We will use irradiation as a model of bone marrow depletion when studying the immune system. This is preferred over the administration of antibodies. We split the dose of irradiation into two exposures as this reduces the risk of irradiation sickness.

When looking at metabolic markers we may perform a glucose or insulin tolerance test. This involves taking a small blood sample over a two-hour period following the administration of glucose or insulin. This is the most refined in that the animals metabolism will process both substances naturally over this period of time.

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

Whilst lower order animals such as fish and flies are useful models of some aspects of disease then they do not have a fully functional immune system. Therefore, experiments in a mammal are critical for relevance to human disease and mice represent the lowest order of mammal that can be used to achieve our aims as they provide a good representation of the human system (sharing over 90% of their genes).

We cannot use terminally anaesthetised animals as we need to induce disease and then allow it to develop.

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

For surgical models, long-acting (e.g. opioid) analgesia will be given to alleviate any pain immediately post-operation. All animals that have undergone a procedure will be carefully monitored by staff trained to work with the models performed. Any animals displaying signs of pain such as piloerection, paleness and changes in mobility will be humanely killed.

Wherever possible we will administer substances for experimental purposes in food or drinking water, instead of injections. Where daily administration cannot be done in food or



water the use of a surgically implanted miniature infusion pump will be considered over injection routes. If injection routes are necessary, we will choose the route with minimal harm but will still enable the maximum delivery to the tissue target.

Where knockout of molecules of interest is required, conditional knockout in specific cell types is preferable to global deficiency, to focus the reduction of expression in our immune compartment of interest and minimise off-target effects.

The use of adjuvants is necessary in some of our models, and we will use those adjuvants that cause the least severe response. Where we need to use an adjuvant that will cause a more severe response, we will deliver the dose at two sites to reduce the risk of inflammation at a single injection site.

For bone marrow transfer experiments, irradiation will be given in 2 separate doses - at least 3 hours apart - to minimise side effects. We will also provide antibiotics prophylactically to prevent infection as advised by NVS.

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

We will follow PREPARE and ARRIVE guidelines of best practice when designing and reporting experiments under this licence. The LASA guidelines will also be consulted.

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

We will attend welfare meetings and 3R's meetings held at the establishment, and sign up to the NC3R's website updates. We have joined the NC3R cardiovascular network. We will interact with the regional NC3R's representative, as well as the Named Information Officer, and disseminate acquired information in our group lab meetings. We will keep up to date with scientific literature and collaborate with others in our field to ensure we are using the most refined methods for our disease.

A retrospective assessment of refinement will be due by 6 February 2030

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. Aquatic Ecotoxicology

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

Environment, Regulatory, Toxicological, Fish

Animal types	Life stages
Zebra fish (<i>Danio rerio</i>)	juvenile, embryo, adult
Rainbow Trout (<i>Oncorhynchus mykiss</i>)	juvenile, embryo
3-Spined Stickleback	juvenile, embryo, adult
Medaka (<i>Oryzias latipes</i>)	juvenile, embryo, adult
Brown Trout (<i>Salmo Trutta</i>)	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?

To provide toxicological data that government and international regulatory agencies have stipulated necessary for a balanced assessment of the potential impact of new and existing chemicals on the aquatic environment and its ecology (e.g. fish). The regulatory guidelines are published and manufacturers are obliged by law to provide such data for two reasons (1) Safety of the environment and/or (2) Human health



A retrospective assessment of these aims will be due by 19 February 2030

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 introduction of new chemicals, such as drugs, biocides or agrochemicals, is generally considered to be beneficial because of the associated improvements in the quality of life. New medicinal products can alleviate diseases and handicaps, new plant protection products can optimise crop yields and improved industrial chemicals can facilitate production of manufactured materials with greater utility and economic advantage. Although suited for their purpose, it is important to show that these chemicals do not also exert an adverse effect on humans, wildlife or the environment.

The primary benefit to this project is for the facilitation of sound regulatory decisions to ensure that the concentrations of man-made chemicals (e.g. agrochemical, industrial and pharmaceuticals) entering ecosystems (e.g. lakes, ponds and rivers via groundwater and surface water) are safe and do not pose potential risks (e.g. acute/chronic toxicity, bioaccumulation, biomagnification or development/endocrine abnormalities) to ecosystems, food chains, human health and populations.

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

The primary output of this project is the production of data for the facilitation of sound regulatory decisions to ensure that the concentrations of man-made chemicals (e.g. agrochemical, industrial and pharmaceuticals) entering ecosystems (e.g. lakes, ponds and rivers via groundwater and surface water) are safe and do not pose potential risks (e.g. acute/chronic toxicity, bioaccumulation, biomagnification or development/endocrine abnormalities) to ecosystems, food chains, human health and populations.

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

The introduction of new chemicals, such as pharmaceuticals and pesticides, is generally considered to be beneficial because of associated improvements in the quality of life (e.g. a new anti-cancer drug saving lives / growing healthy crops to provide maximum yield for consumption / ensuring veterinary medicines used in livestock do not taint meat or pass into humans via consumption). Although suited for their purpose, it is important to show that these chemicals do not also exert an adverse effect on humans, wildlife or the environment.



The introduction of new chemicals to replace those considered harmful to the environment is encouraged, however, this is dependent on the generation of reliable toxicological data for comparison purposes and to demonstrate the benefits whilst ensuring secondary or non-target exposure are minimised to safe non deleterious levels.

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

Production of high quality scientific data/reports for use in regulatory environmental risk assessments. Although the study data and scientific reports are the intellectual property of Sponsor companies, non- study specific information may be shared with industry to further optimise study set-up/conduct (eg testing of difficult substances/mixtures).

Publication/presentation of posters/papers or oral presentations, as applicable, either at conferences (eg SETAC) or in collaboration with other groups (eg NC3Rs) based on experiences with the studies conducted.

Species and numbers of animals expected to be used

- Zebra fish (*Danio rerio*): 30775
- Medaka (*Oryzias latipes*): 140450
- Brown Trout (*Salmo Trutta*): 245
- Rainbow Trout (*Oncorhynchus mykiss*): 35440
- 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.

The test species used on studies are recommended for testing in the regulatory guidelines and have been selected as appropriate on the basis of practical and scientific criteria; e.g. availability, size, practicality of maintenance, known sensitivity, economic or ecological importance, regulatory stipulation and representation of trophic level or niches. The life-stage used will depend on the test but include egg, juvenile and adult stages.

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

The individual studies undertaken involve exposure of groups of fish to varying concentrations of chemicals to assess the effect that the chemicals may have on survival and/or growth of the fish. The exposure may be aqueous or via the diet. The most appropriate dosing system will be selected based on the nature of the test substance and test guideline. For aqueous exposures, the test substance will be introduced into the water prior to the addition of the fish. Where dietary exposure is required, the test substance will be mixed with the diet prior to feeding the fish.



If genetic sex determination is required for a test, a small clip will be taken from the caudal or anal fin. This procedure is conducted without anaesthetic as experience has shown that with suitably trained staff this can be conducted quickly and efficiently. This reduces potential harm and stress to small fish exposed to anaesthetic.

The duration of exposure depends on the study type conducted and can range from 96 hours to several months.

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

Fish may experience various effects ranging from mild changes in colouration or abnormal swimming behaviour to severe symptoms including lethargy, moribundity and even death. Dependent on the test conducted, mild or moderate symptoms may be observed for the duration of the test. Fish observed to be moribund, exhibiting total loss of equilibrium (overturned at surface/base of vessel or barrel rolling/spiral swimming) or are unresponsive to external stimuli will be removed and humanely killed.

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)?

Adverse effects ranging from mild discomfort through to death are expected during the project. Over the course of the project approximately 15-20% of the fish tested are expected to experience severe symptoms. However, in the majority of exposed fish adverse effects will be mild.

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

- Killed

A retrospective assessment of these predicted harms will be due by 19 February 2030

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?

Central governments have imposed the obligation for industry to use animals for this scientific purpose and at present no alternative approaches are acceptable or available.

The evaluation of environmental risk considers the hazard (toxicity) and the potential exposure. The hazard is the inherent toxicity of the chemical that reflects the susceptibility of the species and is used to estimate the susceptibility of the overall population or ecosystem. The potential risk of exposure includes the intensity, duration and probability of chemical exposure to the environment. The European requirements for aquatic ecotoxicology testing are laid down in legislation such as the Plant Protection Products Directive EC No 1107/2009, the Biocides Regulation No 528/2012, the REACH Regulation No 1907/2006 and Directives 2001/82/EC and 2001/83/EC regulating veterinary and human use medicinal products, respectively. The European Medicines Agency (EMA) are concerned with the requirements for environmental effects testing of pharmaceuticals in the EU. There is also similar requirements in other major jurisdictions (US and Japan) including the Toxic Substance Control Act (TSCA) and Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) passed by US Congress and Japan Chemical Substance Control Law.

Current regulations e.g. REACH, require the use of fish to assess potential environmental effects. Non- animal alternatives have not yet been fully accepted by various regulatory authorities and hence cannot be used yet to replace animal testing in this context.

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

OECD Guideline for Testing Chemicals No. 236 Fish Embryo Acute Toxicity (FET) Test. This short term test utilises fish embryos and completes prior to independent feeding of juveniles. This test may be a suitable replacement for the acute fish toxicity test (OECD 203) if agreed with regulatory authorities.

OECD Guideline for Testing of Chemicals No. 319A Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP) and No. 319B Determination of in vitro intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9). These guidelines may be used improve in silica prediction of bioaccumulation in fish and hence reduce or replace the requirement for the bioaccumulation test (OECD 305)

OECD Guideline for Testing of Chemicals No. 249 Fish Cell Line Acute Toxicity - The RTgill-W1 Cell Line Assay. This test may be a suitable replacement for the acute fish toxicity test (OECD 203) if agreed with regulatory authorities.



Why were they not suitable?

The aim of acute and chronic toxicity testing is to identify the effect of a chemical on the whole animal under conditions that would mimic exposure in the natural environment. Therefore, the use of in-vitro tests in many instances is not appropriate. However, as science continues to improve, different stages such as eggs, sperm and cell cultures are being investigated as alternatives to whole animal testing, we will continue to monitor progress in this area and will be proactive in suggesting alternative test designs to Sponsors. However, this will require the acceptance by regulatory authorities and alternative guidelines to be accepted and developed for regulatory purposes. Currently both the OECD No. 319A and 319B have been validated at Smithers.

A retrospective assessment of replacement will be due by 19 February 2030

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 estimated numbers are based on the maximum number of fish required by each regulatory test guideline and the estimated number of each test type likely to be conducted over a 5 year period (based on previous year's data). In addition, knowledge of potential regulatory guidance document changes (for example, changes to pharmaceutical and REACH regulation requirements) have been considered.

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

The number of fish used in regulatory ecotoxicology studies is specified in the relevant test guidelines and is the minimum that is sufficient to allow meaningful data interpretation and submission to a range of regulatory authorities. The use of the specified numbers of fish ensures that the data generated will be acceptable to regulatory authorities and hence the need for subsequent duplication or supplementary testing.



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

Where possible the results of the computer-generated predictions, physico – chemical testing and non- animal tests will be used to aid in the prediction of toxicity hence reducing the number of animals required to satisfy the regulatory requirement.

Where information on a chemical is available which suggests that there would be no or limited toxicity then a limit test may be performed for an acute or chronic test. Alternatively, if data for aquatic invertebrate and plant are available, a threshold approach based on these data may be appropriate for acute fish testing. This approach is used to demonstrate that fish are the least sensitive of the tropic species and is equivalent to a toxic limit test.

If range-finding tests are required, these will be conducted using minimal replication and reduced animal numbers. Where data are available from another fish species, it may be possible to use this information to determine a concentration range to be used for a definitive test and therefore, there would be no requirement for a range-finding test to be conducted.

For more complex studies, for example life-cycle studies, any data available will be evaluated in order to design the most appropriate study to ensure the minimum number of fish are used whilst providing the maximum amount of relevant data to ensure further fish studies, or repeat of studies, are not required. Where appropriate, the study protocol will be submitted to the relevant regulatory authority to ensure suitability.

In addition to the above, where possible, shared control groups will be used when acute toxicity tests are conducted at the same time on the same species to reduce the numbers of fish used.

A retrospective assessment of reduction will be due by 19 February 2030

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 fish species and life stage(s) used have been selected in accordance with the relevant regulatory Test Guidelines and the age ranges of the fish are such that they are of the lowest neurophysiological sensitivity that will allow evaluation of the specific endpoints. Typically, the recommended species tend to be those that have a greater sensitivity to chemicals, which effectively safeguards less sensitive species in the environment.

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

The aim of acute and chronic toxicity testing is to identify the effect of a chemical on the whole animal under conditions that would mimic exposure in the natural environment. Therefore, the use of in-vitro tests in many instances is not appropriate. However, as science continues to improve, different stages such as eggs, sperm and cell cultures are being investigated as alternatives to whole animal testing, Smithers will continue to monitor progress in this area and will be proactive in suggesting alternative test designs to Sponsors. However, this will require the acceptance by regulatory authorities and alternative guidelines to be accepted and developed for regulatory purposes.

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

Frequency of the observations may be increased if moderate effects are observed, for example, hyperventilation, lethargy, positional disorder, to monitor the severity of the symptoms and hence reduce potential suffering if symptoms become severe and require humane killing of the fish. Where an animal displays significant effects and distress following exposure to a chemical (eg moribund, exhibiting total loss of equilibrium (overturned at surface/base of vessel or barrel rolling/spiral swimming) or are unresponsive to external stimuli), it will be removed from a test and humanely killed.

Animals will be humanely euthanised using an overdose of anaesthetic or concussion followed by spinal cut and destruction of the brain. The anaesthetic solutions (MS-222) are pH adjusted to reduce irritation to the mucus membrane.

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

Studies are conducted in accordance with the OECD Test Guidelines (<https://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm>)

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



Care will be taken (through monitoring of the OECD website and in conjunction with the Smithers regulatory group) to ensure the most up-to-date test guidelines are followed. In addition, members of the Smithers group are actively involved with workshops/focus groups initiated by the UK NC3Rs organisation and conference attendance (eg SETAC conference, Akademie Fresenius conferences).

A retrospective assessment of refinement will be due by 19 February 2030

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. Avian Ecotoxicology Studies

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

Avian, Environmental, Risk Assessment

Animal types	Life stages
Duck or other waterfowl	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?

The purpose of this work is to examine the toxicity of chemicals, agrochemicals, microbial pest control agents (MPCA) or other substances to evaluate any harms that may result from exposure of birds to such substances in their natural environment.

A retrospective assessment of these aims will be due by 16 February 2030



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?

Governments require and the public expects that substances which may enter the natural environment are safe or their hazards well understood. This necessarily involves the conduct of whole-animal toxicity tests such as those included in this project, which form part of a framework of studies designed to investigate potential effects on all components of the ecosystem. These tests are required by Global Regulatory bodies. Most studies are run to specific designs outlined in the relevant test guidelines.

Birds represent a major group of terrestrial vertebrates which may be at risk from exposure to environmental pollutants, and so that risk must be adequately characterised and evaluated in order to protect wild bird populations from unacceptable adverse effects.

Tests will only be run in the species required by the individual test guidelines, and/or as required by the regulatory body involved.

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

The principal benefit of the project is the provision of safety data to facilitate risk assessment and sound regulatory decisions on the development/approval of new substances to which wild bird populations will potentially be exposed.

By this process, the safety profiles of products under development can be optimised, and unsafe products identified and eliminated, thus contributing to the protection of the environment.

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

Our customers will benefit, as the data we generate will allow them to progress their substances under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain marketing authorisation.

The project aims to test chemicals (e.g. agrochemicals, biocides and industrial chemicals) and other environmental pollutants in avian species (quails, ducks and other waterfowl species, and passerines such as finches); this will benefit these species, and the taxonomic/ecological groups they represent, in their natural habitat by contributing to the development of safer chemicals and eliminating unsafe ones.



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

The work will be shared with customers who will use it to determine their future strategy, or for submission in documents required by regulatory authorities. Whilst we have no direct control over what happens to the data after we have shared it, we trust from information given to us that it is used for, or to support, regulatory purposes (e.g. to show that a certain chemical is safe for avian exposure).

Where appropriate, we collaborate with our customers to share data we have produced in the form of Scientific publications that are in the public domain.

We are able to advise our customers on which studies are required in their development programme and on suitable study designs, based on our experience and on knowledge gained from previous post- registration feedback from customers and/or regulators, leading to focussed and effective studies.

It is difficult to predict how the benefits of any work done on this project will be seen in the future due to confidentiality issues. However, this work will contribute to the safety of chemicals that avian species are exposed to.

Species and numbers of animals expected to be used

- Other birds: 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.

Birds (quails, ducks and other waterfowl species, and passerines such as finches) will be used, both adults and juveniles. Both are susceptible to the ecotoxicological effects of chemicals.

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

Since ingestion is the most significant route of exposure to environmental pollutants in wild birds, most birds in this project are dosed with the substance under evaluation by direct oral administration (liquid or capsule administration) or by dietary incorporation. Inhalation exposure can also be important (e.g. crop spray drift) and in a small proportion of studies birds may be exposed in whole body inhalation chambers or, in the case of microbial agents (microbes used in crop protection strategies), treated by direct administration into the upper respiratory tract. A small proportion of studies may also require blood samples to be taken or that birds are confined in small cages for short periods to allow the collection of excreta. No significant adverse effects as a result of any of the applied dosing or



sampling techniques are expected (other than transient mild discomfort, e.g. as a result of needle puncture for blood sampling).

Birds used in this project will be humanely killed at the end of the study for post mortem investigations and tissue collection purposes.

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

Most birds used in this project will experience no, or only mild, adverse effects attributable to exposure to test substances (around 92% of birds were classified as having no adverse effects (subthreshold) or mild adverse effects under the last project). However, some birds may experience moderate effects (for example inappetence and weight loss), and in the case of acute/subacute studies, severe signs of toxicity and/or mortality are to be expected in a proportion of birds. Clearly defined humane end-points based on clinical signs are applied and birds humanely killed to prevent unnecessary suffering.

When taking blood, the degree of pain or discomfort an animal feels is considered similar to what a patient would feel having a blood sample taken by a doctor or nurse.

Most birds confined temporarily in an excreta collection cage, or in a whole body inhalation chamber will get used to their new surroundings within a short time and show no adverse effects; however, in a small number of cases, some animals may show a degree of stress or anxiety unless the confinement is discontinued. In some cases the excreta collections can be made in the birds' home cage.

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)?

On the last project, around 12% of the birds used experienced no adverse effects (subthreshold) and 80% of birds experienced mild severity, with around 2% experiencing moderate and 6% experiencing severe adverse effects. The severe effects are almost exclusively seen in the acute/subacute studies, where little information is known about test compound exposure levels (often these are first time in an animal study).

Although some variation in the proportion of severities is to be expected on a service licence like this (where there is a degree of unpredictability in the types of new study requests that will be received and in the toxicity of test articles that will be submitted for evaluation), it is anticipated that the overall distribution of actual severities will be similar to those in the last project as indicated above.



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

- Killed

A retrospective assessment of these predicted harms will be due by 16 February 2030

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 evaluate adequately the effects on wild bird populations of exposure to a given substance in the environment, it is necessary to conduct whole-animal safety/toxicity tests; currently, no scientifically, ethically or legally acceptable non-animal alternatives that would achieve this objective are available.

The regulatory requirements are mainly for UK/EU regulators, but occasionally other regulators in other countries like the US for example. If the requirements for these non-UK/EU tests are over and above the requirements for a UK/EU regulators, or the test required is more severe, then we consult the Home Office to ask for prospective authority to run such tests.

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

None were suitable.

We maintain a constant awareness of regulatory guidance and ensure that where non-animal methods exist which fulfil the regulatory requirement, they are used in preference to animal studies. Currently, however, there are no other non-animal alternatives for the work being undertaken on this project. The regulations we are following will not allow safety decisions to be made on non-animal systems alone.

In vitro and in silico methods (test tube or computer work not using animals) are used in combination with animal studies to inform study designs and assist in understanding of potential toxicity but cannot yet replace in vivo (animal) studies.

Why were they not suitable?

This is because cells in test tubes cannot replicate the complex interactions that take place within a whole living animal and as we cannot model substance exposure in the laboratory



we need to use animals to provide these complex data, which is required for regulatory purposes.

A retrospective assessment of replacement will be due by 16 February 2030

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 proposed are based on levels of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received from customers in the past, and on current enquiries for future work.

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

Studies are designed to provide maximal data and statistical power (where appropriate) from the minimum number of animals considering that it is better to increase the number of animals used to achieve the objective than to use too few animals and risk having to repeat the study.

Whenever possible, common species of animals are used such that a large amount of control background data is available. This reduces the need for large control groups.

All available information is reviewed to ensure each specific test is justified and is optimally designed using appropriate treatment levels. Numbers of birds used in each study are generally linked directly to those in published regulatory guidelines; where non-standard test protocols are required, statistical design principles and relevant experience from previous studies will be used to limit group sizes to the minimum commensurate with meeting study objectives.

In addition, the tiered/sequential approach generally adopted for development/safety evaluation programmes (see also below) enables the results of initial testing (usually single dose/acute exposure tests) to be used to minimise the subsequent tests in the programme, either through provision of a basis for appropriate dose selection, or in some cases to determine whether a subsequent study is undertaken at all (either through



regulatory/risk assessment triggers or because it may demonstrate unacceptable toxicity and halt the testing programme).

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 try to get as many outputs as we can from a single animal where possible, without adversely affecting its welfare. So, if we need to take several different samples, for example, we will often do that in the same animal, rather than using separate ones, when possible.

Before our main studies, where appropriate, we use smaller groups of animals to get an idea of the doses we need to use for the main studies. Where they are considered necessary, these preliminary studies are important as they give us confidence that the doses we are using are correct prior to testing them in bigger groups of animals as required by global regulators.

A retrospective assessment of reduction will be due by 16 February 2030

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.

To prevent unnecessary pain/suffering and to refine studies/data requirements in any given development programme, a tiered approach to testing is adopted; this enables the results of initial testing (usually single dose/acute exposure tests) to be used to refine the subsequent tests in the programme by enabling appropriate dose selection, or through identification of specific effects on which subsequent tests can be focussed.

Birds in all studies are observed regularly to monitor condition, behaviour and clinical health. Bodyweight and food consumption, both useful indicators of wellbeing and health, are also monitored. Appropriate humane end-points are applied to minimise adverse effects while achieving study objectives; severe effects are not allowed to persist, and the affected birds are promptly humanely killed where necessary to prevent further suffering.

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

The birds used on these studies are specified in the regulatory guidelines for these tests. The tests would not be acceptable to Global Regulators if other species were used.

Due to the length of some dosing and procedures needing to be performed, it is not practical to perform them under terminal anaesthesia.

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

During dosing and restraint, animals are constantly and closely watched for signs of distress. If equipment is used to enable us to achieve the scientific aims of the study (e.g. confinement in a metabolism cage for excreta collection), then we would habituate animals to this equipment prior to dosing where appropriate. We would remove them from the study in the unlikely event of them not habituating to the equipment.

All procedures are subject to ongoing assessment and technique improvement and we participate in cross-company working parties on best practice. Animals are regularly reviewed for general health and veterinary staff are on call at all times to assess any adverse events and provide supportive care and treatment as appropriate.

If we have to repeatedly withdraw blood using a needle and syringe, we would choose different sites to do this where possible to minimise local adverse effects. Where appropriate we may place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. Similarly, if we need to dose repeatedly by injection then we try to use different sites to minimise local adverse effects. Of course, everyone who performs these procedures is trained to a high standard.

Refinements to improve the animals' experience include but are not limited to group housing and appropriate environmental enrichment, including provision of perches, substrates for dust bathing, objects to encourage pecking/foraging behaviours, provision of water to ducks for bathing and food items to encourage foraging/ dabbling behaviours etc.

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

OECD (2016). OECD Guideline for Testing of Chemicals 223: Avian Acute Oral Toxicity Test. OECD (1984). OECD Guideline for Testing of Chemicals 205: Avian Dietary Toxicity Test.

US EPA (1996). Microbial Pesticide Test Guideline OPPTS 885.4050: Avian oral, Tier I.

US EPA (1996). Microbial Pesticides Test Guideline OPPTS 885.4100: Avian Inhalation Test, Tier I.



US EPA (1998). Guidelines for ecological risk assessment. USEPA/630/R-95/002F. United States Environmental Protection Agency, Washington DC, USA.

US EPA (2012). Ecological Effects Test Guideline OCSP 850.2100: Avian acute oral toxicity test. US EPA (2012). Ecological Effects Test Guideline OCSP 850.2200: Avian dietary toxicity test.

UK Home Office (2005). The conduct of Regulatory Toxicology and Safety Evaluation Studies.

EC Regulation 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market.

Hart et al (eds.) (1999). Avian Effects Assessment: a Framework for Contaminants Studies. Report on a SETAC workshop on 'Harmonised Approaches to Avian Effects Assessment', Woudschoten, the Netherlands.

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement: Removal of blood from laboratory animals and birds. Laboratory Animals, 27, 1-22 (1993).

Fifth report of the BVAAWF/FRAME/RSPCA/UFAW joint working group on refinement: Laboratory birds: refinements in husbandry and procedures. Laboratory Animals, 35 Suppl 1 (2001)

Fair, J., E. Paul, and J. Jones, Eds. 3rd edition: Guidelines to the Use of Wild Birds in Research. Washington, D.C.: Ornithological Council (2010).

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

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animal Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

A retrospective assessment of refinement will be due by 16 February 2030

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. Understanding the signals that govern tissue development, regeneration and 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

Liver, Kidney, Cancer, Regeneration, Development

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

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?

For organs to form and grow in the embryo cells need to be able to sense and perceive their environment. They do this by receiving special signals from other cells. The same is true when an adult tissue grows or a cancer forms. By understanding how cells within tissues communicate to each other in health we aim to figure out what goes wrong with this communication during disease and cancer.



A retrospective assessment of these aims will be due by 19 February 2030

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 number of patients living with liver and kidney disease (approximately 2 million and 3 million respectively) is increasing in the UK and for some patients this leads to a much higher chance of them getting cancer in these organs, in fact these patients are 1000-times more likely to develop cancer. To develop new medicines to treat both liver and kidney disease and cancer we need to understand how organs grow when they are healthy and figure out what goes wrong during disease. We work with mouse and rats with liver and kidney diseases to find out how cells talk to each other and then use new types of medicines to interrupt this and measure whether this improves the ability of the liver and kidneys to work normally.

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

By the end of this project we will generate new knowledge and understand:

- How specialised, tube-shaped structures in the liver called bile ducts grow in the foetus and have found out how these structures re-grow in the injured adult liver.
- What causes cysts to form in the ducts of the liver and tubules of the kidney and asked whether we can stop this.
- What happens when a cancer starts to form in adult organs and determine whether we can stop the transformation of normal cells into cancer.

We will publish our work so that other scientists can read about our progress. We also work closely with a number of UK and European charities who will help us communicate our findings to patient groups and care-givers.

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

In the short to middle term our work will benefit the scientific community by providing new data and information of how livers and kidneys become diseased and how cancers form, particularly in the context of a diseased organ. Our new information will not only be used by people working in these organs, but will be important for scientists working in tissue regeneration and cancer more widely.



In the longer term, we design our research to improve the lives of patients with liver and/or kidney disease. With this in mind we always ensure that our animal models reflect human disease as closely as possible with the hope that this will improve the translation of our findings into patient treatment. Our previous studies have led to new medicines being tested for liver cancer.

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

We maximise the outputs of our work using three approaches.

We work closely with charities in the UK , Europe and the US to distribute our new knowledge to both the medical and scientific communities as well as to patients, caregivers and families.

We are part of the leadership group for an EU-funded network which brings together scientists and doctors within this field to accelerate discovery. As part of this network we regularly meet to discuss data, new models and ideas and within this network also exchange unpublished data and resources.

We publish our data on pre-print servers so that our data is can be found by anyone (i.e. is open access) and so there are no barriers to accessing the data we have generated with animals. In addition to this, depositing our findings on these platforms enables us to disseminate our work more quickly and potentially to a wider audience.

Routinely in our manuscripts we include negative data - where we have tested a hypothesis using animals and which has not shown to have an effect or phenotype. It is important to us that this is either included in the publication or deposited alongside pre-prints to ensure that experiments (particularly those using animals) are not unknowingly repeated.

Species and numbers of animals expected to be used

- Mice: 41,200
- Rats: 1,100

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.

Mice and Rats will be used within this project and we are interested in understanding health and disease over the course of a lifetime. Mice in particular have become a very powerful model in understanding how diseases progress as we can make small changes to their DNA and ask whether this makes a disease better or worse. Rats are often



valuable for modelling more slowly progressing diseases such as cancer and in our work more closely reflect the human pathophysiology of the human cancer.

For our work we need mice and rats to have normal immune cells (the cells that normally deal with bacteria and viruses) as these special types of cell are also involved in the progression of disease and cancer.

- For our work looking at how organs form and grow in the foetus we will use mice that have not yet been born. These will normally be from late-stage pregnancies once the foetus has developed organs.
- In studies where we are investigating the genetic causes (due to changes in our DNA) of disease, such as polycystic liver and kidney disease we will use juvenile and adult stages to determine how disease progresses and develops throughout infancy and into adulthood.
- We use adult mice to investigate how cancers form and determine whether certain experimental medicines can be used to stop cancers from forming and growing.

We choose these life stages as they most accurately reflect the natural disease history in humans. Cancer for example is typically a disease of older adults, using juvenile stages would not be appropriate.

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

The majority of the animals we use are genetically altered and are treated with toxic compounds to give them liver disease or liver cancer (some of our animals do not require toxic compounds, such as animals that we have developed to understand genetic diseases like polycystic kidney and liver disease). Generally these compounds are given in their drinking water or in their food.

Once an animal has a disease we often try to treat it with experimental medicines (approximately half of our animals will be treated this way) and normally all of our procedures are concluded within 6 months, though rarely it's important to have small groups of animals who are older (18 months), but with milder disease. We normally give our experimental medicines orally, but sometimes it is necessary to inject the animals. Depending on the medicine animals could be given a dose daily - we take this into consideration when deciding how we give the medicine.

Typically an animal will not experience a surgical procedure, however a very small number (around 1% of our animals) will. For these animals they will normally receive a laparotomy (where a small incision is made in the abdomen) and then will either have a small suture tied around their bile duct to force it to regenerate and regrow or will have cancer cells injected into the liver as a way of exploring how liver cancers grow.



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

For many of our studies, such as those studying how the liver forms in the foetus and how polycystic liver and kidney diseases grow there are very few to no adverse effects.

In animals with liver disease we often see that animals lose weight (this is our principal measure of how disease is progressing), however we have developed a clinical scoring sheet to ensure that the adverse effects of having liver disease can be closely monitored and the experience of the animal is assessed against this clinical scoring sheet. In addition to weight-loss, very rarely animals may develop jaundice and/or ascites (fluid in their abdomen) which can make breathing and activity more difficult; the development of these clinical signs would score highly on our clinical scale and would result in the premature termination of the study.

For animals with cancer, again we have developed a panel of clinical signs that we adhere to to ensure that the adverse experiences of the animal are minimised. These signs could include general symptoms of being unwell including loss of social interactions, failure to groom and withdrawal from food, but might also include other cancer-specific signs such as their abdomens growing and becoming darkened. Through our long-term experience of using these models we have been able to develop a scoring sheet which ensures that the actual severity experienced by an average animal is monitored and not allowed to escalate beyond what we expect.

We have included time limits in our protocols to ensure that animals are not experience significant clinical signs for extended periods of time and that animals with liver disease and cancer do not progress to "end-stage" disease, a point where their consciousness may be reduced and they are living in chronic pain.

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)?

Within this license we have a mixture of mild, moderate and severe protocols. 20% of animals (mice) will be held on a mild procedure (normally breeding and maintenance). Rats will not be bred in house, rather they will be purchased from a licensed breeder. The remaining 80% of animals (both mice and rats) will be moved onto moderate or severe protocols. The reality is that within this 80%, the majority (>90%) will only experience moderate severity - some, the remaining 10% might experience an increased severity, mainly driven by weight-loss, however we have introduced a number of mitigations to reduce the number of animals experiencing this severity.



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

- Killed

A retrospective assessment of these predicted harms will be due by 19 February 2030

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?

How tissues form and change during disease is a complex process that involves many different types of cells.

While cells in a dish can reflect some of these processes, generally they do not reflect the complexity of tissues. Diseases often progress and become worse over time, which can increase the risk of cancer. Modelling disease progression is simply not possible using non-animal alternatives.

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

Wherever we can, we perform experiments with alternative to animals. This normally involves using primary patient tissues or cells derived from human or animals which are immortalised. These fall into two main categories:

1. Precision cut tissue slices: These are thin live slices of liver or kidney tissue normally from human (though we can use mouse and rat tissue for this). These slices can be put in dish where they maintain their viability for a number of days and can be used to test medicines "at scale". The benefit of this system is that from a single biopsy we can generate many 10s of slices, meaning we can perform a larger number of experiments from a single samples.
2. We regularly use human cell lines or organoids. Cell lines are normally cells taken from a patient and grown immortally in a dish. They are quick and easy to grow; however do not often reflect the cells that originally contributed to their development. A major, recent advance is organoids which are patient cells or tissues that are grown in 3D and normally more closely resemble the tissue or origin. These can be grown in a dish too and can be used to test medicines or understand how genes/proteins work. We have a biobank



of human and mouse organoids which means that we can replace some of the experiments we would have historically done in animals with these tools.

Why were they not suitable?

The non-animal models we use are excellent, simple systems; however they do not replicate the progression of disease and the complex multicellular nature of regenerating or cancerous tissues. It is important for us to have a functioning immune system, complex extra-cellular matrix that evolves overtime and multiple other cell types. Using our in vitro models replaces some of the animals we use in research, but at the moment they are insufficient to replace them completely.

A retrospective assessment of replacement will be due by 19 February 2030

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?

Over the last 10 years we have performed a range of experiments where we have used animals to understand how the liver forms, regrows following injury and becomes cancerous. We have developed many of the tools and methods we use in our lab and because we know how animals respond to these models we can accurately determine how many animals we need to use in each experiment to test whether a medicine will be effective, for example. This strategy enables us to use the minimum number of animals to get robust scientific answers to our questions.

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

During the development of our protocols we have reduced animals by optimising our experimental protocols over a number of years and performing pilot studies to understand what the variation of a particular protocol is between animals. We also use experimental design software to help us ensure that we are using animals efficiently and effectively within our procedures.



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

We regularly share tissue with other groups locally, nationally and internationally, this limits the number of animals required for our studies. Similarly we ensure that our breeding protocols are as efficient as possible. This is a particular challenge as many of our mice are complex mutants with changes in a number of genes, however wherever possible we breed our mutant mice to reduce the number of pups that are born which are not experimentally useful.

The vast majority of our animals are maintained as frozen sperm or embryos to limit genetic drift from expansive, long-term breeding.

A retrospective assessment of reduction will be due by 19 February 2030

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.

In this project we use mice and rats to understand how organs develop and change as they grow and become diseased. Over the last 15 years we have worked to develop protocols that minimise suffering and pain to our experimental animals as they develop liver disease and cancer. Typically the way we generate diseases in these animals is by providing toxins in their food or water although sometimes, in a small number of animals, we use surgery to cause a disease. On this application we will include the use of transgenic and mutant mice which are genetically amenable and easy to house. There is a pre-existing "library" of mutant mouse lines that we will make use of in this project and in the past we have had excellent results in dissecting disease by modulating gene function in specific cell types.

Rats will be used mainly to model bile duct cancer (cholangiocarcinoma) and we have developed an in vivo model of this process. The benefit of this model over our mouse



models is that it more closely reflects the processes which patients go through as they develop bile duct cancer.

At all times we use the least invasive protocols possible, if we are giving medicines we normally give these orally rather than through injections. If animals are having an operation they are given anaesthetic and close post-operative care to ensure that there is no or very limited lasting harm.

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

Where we can, we use early mouse and rat life stages such as embryos and fetuses.

For the majority of our work we are interested in understanding how disease and cancer develops over time. To do this we need to use animals in infant, adult and aged parts of their lives to understand how these processes progress. Less sentient organisms do not have the same tissue structure as mammals and modelling liver and kidney disease using these alternatives is not currently feasible.

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

Over the course of our previous license we have tried to make the liver disease and liver cancer models we use the very best we can. We have introduced a high protein diet to mice and rats that have established cancer as we have found that this prevents some of the weight loss seen in these animals. We have also established a score to look at how animals' appearances have changed over the time of disease and cancer. This way we have a record of any changes and know what to look out for in future experiments.

As we develop new models we will continue with these principles and adapt our scoring system and monitoring protocols to ensure that our methods are always the most refined. We will work with other research groups to adapt and disseminate our protocols to promote a community-level approach to refinement.

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

NC3Rs guidance and PREPARE guidelines as well as engaging with other groups on their best practice.

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

Our establishment is very proactive in distributing information relevant to the 3Rs, including seminars and workshops held either locally or by third parties such as NC3Rs on a range of topics such as husbandry and handling. This information will be digested and incorporated into the project where possible. I am also a member of our AWERB and we regularly see presentations from local and regional researchers on best practice.



More actively, we engage with the NC3Rs website to stay up to date with best practice and development in animal methodology.

A retrospective assessment of refinement will be due by 19 February 2030

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?

20. Promoting tissue protection and regeneration by targeting endogenous mechanisms

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

Tissue protection, Repair, Heart, Liver, Lung

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 overall aim is to understand the body's own mechanisms that protect tissues from damage and promote repair. We will also develop new treatments to target these processes to address diseases for which currently there are few or no treatments.

A retrospective assessment of these aims will be due by 16 February 2030

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?

We will address three major causes of ill health and death in the West, heart, liver and lung disease, as well as injuries to bone and muscle. Heart attacks remain the leading cause of death in the world, affecting 9% of people over the age of 60 years. Heart failure affects 64 million people worldwide and

5-year survival remains low at approximately 50%. Fatty liver disease affects about 25% of people in the West. Idiopathic pulmonary fibrosis, a progressive condition of the lungs affects 3 million people worldwide and most people only survive 2-3 years from diagnosis. Fractures constitute a major public health problem. Each year 3.6% of the UK population sustains a fracture, with hip fractures alone costing the NHS £1.7 billion. Whilst most fractures heal uneventfully, certain sub-types are associated with excessive morbidity and mortality and patients would derive great benefit from accelerated healing. Skeletal muscle is often injured at the same time as fractures of the limbs and this results in considerable functional disability even after the fracture has healed. Skeletal muscle is also damaged as a result of inflammatory myopathies and heritable disorders such as Duchenne muscular dystrophy.

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

- Understanding of mechanisms underlying tissue protection and repair
- Development of novel therapeutic approaches
- Publications and presentations

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

Throughout the duration of this project new knowledge gained will benefit the members of the scientific community who are currently working in this field by the publication of papers and from our presentations at both national and international conferences

In the long term it is hoped that our discoveries will lead to new therapeutic approaches that will benefit both clinicians and their patients. The scientific community will continue to benefit from our work in the long term.



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

We have a collaborative approach to our research. We have established several collaborations both within our establishment and other establishments. This includes sharing unpublished data and expertise in models. We ensure rapid dissemination of our results at internal meetings and conferences. We ensure negative results are shared and we have strong track record in publishing high quality papers.

Species and numbers of animals expected to be used

- Mice: 22,000

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.

Mice are widely used to model many human diseases. A wide range of relevant reagents are available for mouse tissues and genetically altered animals can be used to validate key pathways. Due to their ubiquitous use, the mouse models in common use have been extensively optimised and validated to ensure that they model all the key aspects of the human disease.

We exclusively use adult mice as they best emulate the conditions in the human we need to model.

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

Genetically altered mice will be bred using conventional methods. The mutations these mice carry are not expected to cause any welfare issues as many are mutations that are silenced and are only activated once the animal is given a substance, which is normally Tamoxifen, daily for 5 days. Some mice will have cells of interest labelled with a fluorescent protein, which will be visible during imaging procedures.

Our project involves causing injury to the heart, liver, lungs, haematological and skeletal systems and then using our therapeutic compound to see how it effects the tissue repair pathways.

For the heart injury model mice will undergo one of three types of injury. The first is a surgical procedure to create a heart attack (myocardial infarction) by obstructing the blood supply to the muscle, causing the tissue to die as happens in the human. The second method is again surgical and is where an aortic band is applied to the heart causing pressure overload to the heart muscle and emulating the heart failure seen in patients. Our third method is to affect the viability and function of the heart muscle by administering substances, such as the chemotherapy drug doxorubicin commonly used in patients to



treat cancer, that are toxic to the heart. Some animals will be imaged during the recovery period so that we can assess the degree of damage and recovery. This will be done over a period of up to 6 months. Mice that undergo surgery will be given pain relief following surgery just as a human would.

Some mice will undergo liver injury by feeding them diets that have been modified. This can be either an acute or chronic model. The mice may also undergo a surgical procedure to insert a viewing window in the abdomen so we can image the liver throughout the experiment.

Some mice will be given a substance to induce lung fibrosis.

Some mice will be given a substance such as a chemotherapy agent to induce injury to the hematological system.

Some mice will undergo a fracture of the femur which will be supported with an external splint as in humans with a fracture. The fracture will be monitored using weight bearing exercises and some imaging.

Some mice will be given barium chloride to cause an acute injury to the skeletal muscle so that we can monitor regeneration of the skeletal muscle.

Some animals will be exposed to irradiation to deplete the immune cells so that we can repopulate the immune system with bone marrow cells from a donor mouse. This process is like bone marrow transplant in humans which is used to treat blood and immune systems diseases which affect the bone marrow. Some mice will also be given cells to alter the immune system without irradiation.

Throughout our experiments may make use of blood sampling and imaging to track the progress of the disease. Some mice may receive a cell labelling substance or labelled cells.

At the end of the experiments, which mainly last a few weeks, animals will be humanely killed.

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

Tamoxifen may cause transient weight loss of up to 15% which readily resolves itself once the dosing period is complete.

Heart injury models may exhibit deep abdominal breathing, and in some cases, sudden death due to cardiac rupture and increased mortality due to chronic heart failure as time progresses.



Liver injury models may exhibit weight loss and greasy coat which is due to the modified diets containing high levels of fat. Analgesia is not required for the acute liver injury models as they are not associated with pain.

Lung injury models may exhibit weight loss as well as transient respiratory distress due to administration of the injury substance via intra-tracheal or intranasal route. Analgesia is not administered for the lung injury model.

Skeletal injury model may exhibit some limping and lack of mobility in the first few hours after injection of muscle toxin.

Haematological injury model may exhibit increased susceptibility of infections as well as transient weight loss.

Femur fracture model may exhibit temporary weight loss and limping or lack of mobility for the first days after surgery.

Implantation of abdominal window can also lead to transient weight loss.

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)?

Sub-threshold 33%, Mild 4%, Moderate 50%, Severe 13%.

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 16 February 2030

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?



Wherever possible, we rely on cell-based assays, with a focus on human cells. However, the full spectrum of injury and recovery, including the initial inflammatory and immune response followed by subsequent repair and scarring, can only be modelled in live animals that have a fully functional immune and tissue repair systems. It would not be ethical to perform these studies in humans.

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

We considered the use of cell-based models, including those based on human cells. We also considered using 3D organoids derived from these cells.

We use human cell-based assays to screen potential therapeutics and only proceed with in vivo experiments using those that have proven most efficacious. We also use in vitro experiments using human cells to understand the mechanism of action and use the minimum number of animals to understand the in vivo efficacy and confirm the mechanism of action. Whenever possible, we strive to use in vitro systems to replace animal use. Any replacements made under the current licence will continue to be implemented going forward.

Why were they not suitable?

Cell based assays do not model the full spectrum of the injury response, which includes recruitment of cells such as immune cells from the bloodstream as well as local reparative cells such as stem cells. Whilst 3D organoids comprise multiple cell types, some key pathways such as the immune response, are missing and they are subject to high variability.

A retrospective assessment of replacement will be due by 16 February 2030

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 calculate the numbers we will need based on our knowledge of variability of response between animals, together with the estimated difference we will need to test for statistically significant effects. This has shown we typically require 10-15 animals per group and time point.

We use our annual return of procedures data to estimate the number of animals that we will need to use for breeding.

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

Before commencing a new study, we draw up an experimental protocol, covering our objectives/hypothesis, an experimental outline, detailing experimental treatments, group sizes, the materials to be collected and a detailed method of analysis. We routinely use good laboratory practice aimed at reducing bias, such as randomisation of treatment and blinded assessment of outcomes. The number of animals will be minimised by performing multiple assessments such as non-invasive in vivo scanning on the same animal whenever possible and by using the maximum relevant tissues from each animal. We always aim to derive maximal information from any experimental sample.

All group members have received training in statistical methods and we have consulted statisticians on aspects of our experimental design. Where appropriate, power calculations are performed prior to the start of an experiment to establish the required group size.

Any reductions made under the current licence that will continue to be implemented going forward.

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

Breeding colonies will be managed in line with the best practice guidelines. Particular attention will be paid to genetic stability and good breeding performance. Data from breeding animals are readily available from the in-house database and will be used to make decisions on future breeding stock and to assist in maintaining a suitable colony size to ensure only those animals needed for experiments are produced. Cryopreservation of colonies not required in the short term will be considered.

A retrospective assessment of reduction will be due by 16 February 2030

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 only use models that have been maximally refined and validated by us or other groups in the establishment and elsewhere. Surgical procedures are performed under general anaesthesia, with analgesia administered prior to the procedure. Animals will be provided with soft bedding and moist food on the cage floor when required.

We will use three different models of heart injury, replicating different clinical scenarios. The first is a surgical procedure to create a heart attack. This model is considered the gold standard and most standardised and accurate rodent model of acute cardiac injury in humans. The second method is again surgical and allows to study chronic heart failure and is where an aortic band is applied to the heart and emulating the heart failure seen in patients. Our third method is to affect the viability and function of the heart muscle by administering substances toxic to the heart, such as the chemotherapy drug doxorubicin commonly used in patients to treat cancer.

There is no single injury model that is perfect and that can simulate all liver disease, multiple injury models are required to simulate different acute and chronic liver diseases. We will use two models of liver injury, an acute and a chronic model. This is achieved by feeding them diets that have been modified. The mice may also undergo a surgical procedure to insert a viewing window in the abdomen so we can image the liver throughout the experiment.

We will use one model of lung injury that induces lung fibrosis. Opioid analgesics would depress respiration and non-steroidal analgesics can limit lung fibrosis through inhibition of COX2.

The skeletal muscle injury will be used to monitor regeneration of the skeletal muscle. There are different models available, but the optimal and most refined model is the barium chloride (BaCl₂) model that induces localised muscle injury. This is achieved by injecting a toxin (barium chloride) that leads to the death of the local muscle fibres to cause an acute injury of the skeletal muscle.

The femur fracture model where a fracture of the femur will be supported with an external splint, as would be used in humans with a fracture. The fracture will be monitored using modalities such as weight bearing or imaging.



Some animals will be exposed to irradiation or substances to deplete the immune cells so that we can repopulate the immune system with bone marrow cells from a donor mouse. This process is like bone marrow transplant in humans which is used to treat blood and immune systems diseases which affect the bone marrow. Some mice will also be given cells to alter the immune system without irradiation.

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

We need a fully functional mammalian immune and tissue repair system that emulates the human to achieve our studies which less sentient species such as fish or worms do not possess.

We need to follow the effects of the injuries we create over a period to understand the full spectrum of tissue response which prevents us from restricting our studies to terminally anaesthetised animals.

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

We use the least invasive surgical techniques but we will keep looking at new refined models that allow us to achieve our scientific objectives. For example, for the myocardial infarction model we use a small incision to access the heart and obstruct the artery without displacing the heart. For the fracture model we will use an external fixator that is compatible with serial CT scanning to allow us to follow animals longitudinally. For the thoracic artery constriction model we use a recently described (Liu et al, STAR Protocols 2020, 1(3): 100186) which uses a minimally invasive approach that avoids cutting the ribs and leads to improved survival. We will continue to monitor the literature for additional refinements to all our protocols. We ensure that all animals receive adequate analgesia, monitor animals closely through the perioperative and post operative period as often as is necessary to minimise the harms and use effective Standard Operating Procedures. There is increased monitoring of mice that exhibit any signs of undue suffering, and they are humanely killed if they do not recover within a defined period. We will continue to closely monitor the literature and liaise closely with our collaborators to keep abreast of further refinements and implement them.

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

We will use the LASA guidance for the administration of substances and on aseptic techniques during surgery.

The NC3R's will be consulted where we are looking for best practice in minor procedures and ensuring we follow best practice in terms of the welfare of the animals.

We will follow the ARRIVE and PPREPARE guidelines in ensuring our experiments are carried out in a manner that will allow reproducibility.



We monitor the literature and implement the most up to date best practice.

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

We stay abreast of the latest advances in the field by keeping up to date with the literature and attendance at conferences. We also regularly liaise with our collaborators, who are also experts in the field, to discuss the latest advances in experimental design and methodology. The NC3Rs website provides additional resources. In addition, we attend internal welfare meetings as well as 3R's events held in our establishment.

We will also liaise with the Named Information Officer to assist us in searches to find refinements.

A retrospective assessment of refinement will be due by 16 February 2030

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. Immune responses to new vaccine platforms

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

mRNA treatments, vaccine responses, infection, cancer, toxicity

Animal types	Life stages
Mice	adult, embryo, neonate, 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.

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?

To evaluate vaccine responses and toxicities of new platform-generated vaccines

A retrospective assessment of these aims will be due by 28 February 2030 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 have been big improvements in how well mRNA treatments work lately. mRNAs are special messengers that deliver instructions for our cells to make specific proteins, we can design them to contain these instructions. The proteins produced will help us fight off certain diseases, such as viruses, e.g., severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We can use these designed mRNA for vaccination and/or therapy. We have witnessed the success of mRNA treatments through mRNA SARS-CoV-2 vaccines, which have helped us overcome COVID-19 pandemic. In addition to this, recent work has shown that mRNA treatments are very promising in the development of pancreatic cancer medicines, showing that administration of personalised mRNA therapy targeting tumour proteins, in combination with other drugs, can reduce and delay tumour recurrence in patients. With the recent discoveries showing the promise of mRNA treatments, we hope to develop new mRNA vaccines to protect against infectious diseases as well as cancer. We will need to assess the capability of this and other vaccine platforms (e.g., protein-based vaccines) in mice, the immune response they generate and any potentially harmful side effects they may have before moving to their use in medical treatments.

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

The outputs will be data demonstrating the effectiveness of novel vaccines for infectious diseases and cancer, the identification of harmful side effects if any and the discovery of the best indicators of vaccine success that can be subsequently used in clinical trials to measure responses. It will also generate new information on the underlying mechanisms of vaccine responses. These data will lead to scientific publications, will be disseminated through national and international meetings and may generate Intellectual Property.

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

In the short term, we anticipate scientific publications to arise from these studies, putting together information to show why we should use the findings in real medical treatments. There will also be benefits from identifying those treatments that are not effective or have undesired effects, avoiding future patients from being exposed to non-beneficial, harmful, treatment.

In the longer term, we hope to increase our understanding of vaccination and give reasons why they should be used, in infectious diseases and cancer.



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

The proposed studies require collaboration with pharmaceutical/biotech companies who are developing some of the vaccines we wish to test, and we have long-standing and productive collaborations that will continue to give us access to the best treatments that are commercially viable (and therefore developable).

We will disseminate new knowledge via publication. We would like to publish unsuccessful approaches if there were an appropriate route (e.g., F1000Research), as long as the data were not restricted as part of a commercial agreement.

Species and numbers of animals expected to be used

- Mice: 19040

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.

Mice are the species with the lowest sensitivity of the nervous system likely to produce data predictive of the effect in humans. Also, the majority of animal models to evaluate the immune system and vaccine responses have been developed in mice. This means many resources are already available for mouse models.

Studies of the effects of vaccines on the body and/or on the whole tumour in its complex environment have to be performed in live animals. Vaccination studies usually require adult mice to match the biology of the human disease, in some cases aged mice will be used to mimic vaccination in old people. We will use genetically modified mice to either alter a natural gene of interest or to express a “marker” gene that enables the detection of certain cell types.

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

Approximately 37% of the mice will be used in breeding protocols to produce mice with the correct genes for use in experiments and 63% of the total mice will be used in experimental procedures.

Most of the experimental mice (~77.3% of the total for experimental procedures) will either be vaccinated or be implanted with a tumour under the skin on the flank (or occasionally 2 tumours, one on each side).

Vaccines will be generally administered using the same routes as in humans. They will be directly injected into the muscle (intramuscular) or the skin (intradermally). Other routes of delivering may be used, such as directly into a vein using a needle (intravenously), under



the skin (subcutaneously) or into the peritoneal cavity (the one around the tummy, intraperitoneally). In humans, vaccines are often administered intramuscularly or intradermally and we will primarily explore the use of these routes to refine and increase the clinical relevance of our vaccination protocols.

Vaccinated mice will be monitored for several weeks. Booster doses may be administered 3 to 6 weeks apart. The mice will be killed at the end of the experiments, with blood and tissues taken for subsequent analysis.

In the case of tumour-implanted mice experiments, they will be treated for up to several weeks with one or more anticancer drugs, anti-cancer vaccines, and a small number of mice may have the tumour under the skin removed surgically. Drugs may be administered either by mouth or by injection. The typical duration of study for an individual mouse, from the time of tumour implantation, would be 3 to 6 weeks. The mice will be killed at the end of the experiments, with blood and tissues taken for subsequent analysis to provide data on the effects of vaccines or drugs.

Towards the end of the project, some mice may be infected with viruses or bacteria (~4.3% of the total for experimental procedures) or be generated with naturally occurring tumours (~3.4% of the total for experimental procedures) to challenge vaccine efficacies.

A small portion of vaccinated mice will be infected with specific viruses or bacteria to test the efficacy of the new vaccine platforms for infectious diseases. Daily weight measurements will be performed before and after infection to monitor scientific and welfare endpoints. Some animals will be administered vaccines or certain substances, such as antibodies, to test the role of specific cell types within the host.

The microbiota, the bugs in our gut, may influence the response to vaccination. Mice may be exposed to microbiota stabilisers (e.g., antibiotics) and/or colonisation (faecal transplant or administration of specific bacteria strains by oral route). Blood sampling and non-invasive imaging under anaesthesia at any stage of the protocols may be required. A small number of mice may undergo intermittent or continuous administration of modified diets (e.g. high-fat/high-sucrose or similar), or fasting, to evaluate how the body responds to changes in energy and or nutrition. Mice may need to be bone marrow transplanted (~10.7% of the total for experimental procedures) or aged (~4.3% of the total for experimental procedures) to test vaccine platforms in relevant conditions such as ageing.

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

The majority of experimental mice will experience transient discomfort from agents dosing/administration. New vaccine platforms are not expected to induce more than mild discomfort.



Subcutaneous tumours will rarely cause clinical signs but dissemination throughout the body is possible (most likely to the liver or lungs), inducing clinical signs such as breathing problems, hunched posture, decreased activity, possible immobility, signs of distress and weight loss. Some tumours may ulcerate. After the tumours become palpable they will be checked frequently for signs of ulceration.

Should ulceration appear, it will be closely monitored. An animal will be killed if ulceration appears and is not healing, impedes a vital function (locomotion, vision, excretion, etc), or causes other clinical signs. If this should occur the animal will be humanely killed immediately. The weight of the mice will be monitored throughout the study. It is not expected for this to change significantly, however, possible weight gain as a result of developing tumours may happen. Body condition scoring will be included.

They may also experience some adverse side effects from their anticancer drugs (usually seen as weight loss, diarrhoea, and/or reduction in their normal activity levels). Mice fed a high-fat diet may develop greasy coats which can lead to scratching and skin lesions. Ameliorating measures for itchy skin or lesions, such as nail trimming and/or green clay, will be undertaken. Other potential adverse effects of diet-induced obesity include impaired mobility and development of pre-diabetes.

A few animals will undergo short periods of general anaesthesia (e.g., for imaging). They may experience illness due to the presence of cancer (particularly those with pancreatic tumours), which may include weight loss, decreased activity, distress and build-up of fluid in the abdomen.

During infection, control animals without vaccination may experience clinical signs of infection, such as weight loss, reduced activity and motility, difficulty to breath, and in accessing water and food.

Vaccinated mice will receive multiple treatments which carry a risk of adverse effects.

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)?

73% of the mice are expected to experience mild severity.

25% are expected to experience moderate severity.

No more than 2% severe severity.

What will happen to animals used in this project?

- Killed
- Used in other projects



A retrospective assessment of these predicted harms will be due by 28 February 2030

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 Regulatory Authorities (The Medicines and Healthcare Products Regulatory Agency (MHRA, UK), European Medicines Agency (EMA, EU) and the Food and Drug Administration (FDA, USA)) will not allow agents to be tested in humans until they have been shown to be safe and effective in animals.

Our research program involves exploiting the immune system to aid in vaccination strategies, and this requires the whole organism.

Currently, there are no non-animal alternatives that can be used to accurately predict or mimic the generation of an immune response to vaccination. This is why we need to conduct experiments on adult living mice, to explore the fundamental mechanisms of vaccine responses and identify potential ways to improve them.

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

Whilst computer modelling and non-animal models of immune responses are ongoing and improving, these do not yet begin to replicate the complicated conditions and variables that exist in the immune system (e.g., complete structural, hormonal and cellular interactions).

We have generated a cellular system to test vaccine constructs before starting preclinical experimentation. In this system, we have produced cells to express specific molecules to evaluate immune cell responses. We have tested this system and we can predict which constructs are more likely to induce an immune response when we vaccinate adult living mice. This system, with appropriate validation within this programme, will help to replace animals as a first step for assessing vaccine constructs.

We do perform many experiments in cell lines grown in culture dishes, to screen different mRNA therapies, drugs and combinations to identify those most likely to be effective in mice/humans, and to investigate the mechanisms. We also use more complex mixed cultures— mixing cancer cells with other cell types that in the body may support the growth and drug resistance of tumours.



Why were they not suitable?

We do use complex cell cultures, however, these do not replicate the complicated conditions and variables that exist in the immune system. Once we have identified the best therapy options, we have to test them in the context of the whole animal. For example, it is very easy to kill cancer cells in a dish, but we have to identify those therapies that will not also be toxic to the normal body.

A retrospective assessment of replacement will be due by 28 February 2030

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 base our calculations on our previous data and experience. During the last year of our current licence we have performed experiments similar to the ones we plan for our next licence, this has helped us calculate more accurately the number of animals we will use. We also referred to published data of similar approaches and suggestions from our collaborators and colleagues. Additionally, we have used available online tools such as the Experimental Design Assistant (EDA, <https://eda.nc3rs.org.uk>) from NC3Rs to critique and analyse our experimental design.

We have used these estimates of a typical number of mice per study, multiplied by the maximum number of each type of study that we expect to perform over the 5 years, to estimate the total number of animals to be used in this programme. We have then calculated the expected number of mice on breeding protocols likely to be used to provide the required numbers of experimental mice. We will use approximately 60% of the mice from our breeding protocols and 40% wild type from external sources.

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

We will use statistical tools to reduce the number of animals to use. We plan to control the variability through block randomisation, where some variables (e.g., genotype) are balanced at randomisation. This will help to control the distribution of confounding and predictive characteristics between compared groups. We will also use inbred strains to



control inter-individual variation and standardisation of operative procedures and animal facility environmental conditions (e.g. temperature, diet, cage). When possible, we will perform experiments comparing multiple groups with a common control group.

We plan to use both sexes in our experimental groups, this will help improve the information we can retrieve from them and avoid unnecessary replication of experiments. When possible, a within comparison will be performed, we will sample before any treatment to determine baseline levels and compare to the treatment effect within the same animals.

Microsampling (taking tiny volumes) of blood permits multiple samples to be taken from each mouse for antibody levels assessments, so that measurements can be made at multiple times, reducing the number of mice required.

For complex transgenic mouse lines with multiple genetic alterations (alleles), as many alleles as possible will be carried homozygously - with both gene copies the same, so that each offspring definitely inherits the genetic alteration. This reduces the breeding required to generate sufficient offspring carrying all the required alleles for completion of the goals.

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

Pilot studies will be performed to inform subsequent studies. Besides the use of the Experimental Design Assistant (EDA) from NC3Rs, a Bioinformatics/statistics core facility will provide advice on the study design, power calculations and statistical analysis.

If new breeding colonies are established, we will calculate the number of mice required to produce the number of mice with the correct genotype for the studies and check this against the estimate for that protocol. At the annual review, the total numbers used on each protocol will be reviewed in relation to the number permitted on the licence.

We will use the NC3Rs breeding and colony management guidelines to support our breeding strategies: Breeding and colony management | NC3Rs.

A retrospective assessment of reduction will be due by 28 February 2030

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 only mice. Rodents are the species with the lowest sensitivity of the nervous system likely to produce data predictive of the effect in humans. Mice are an established and reliable model for human immune responses. Consequently, there are a range of methods, reagents and testing kits for mouse immune responses that make them more suitable than other rodent models. In addition, there are well-established and reliable genetically modified models that enable us to study vaccine responses and side effects in detail and depth in mice that are not present in other rodents. Also, the majority of animal models of cancer have been developed in mice, and there is a longstanding literature on cancer biology and how drugs work in the body based on mice, which provides the background to our studies. The use of these well-documented models means that adverse effects can be treated early and more appropriately mitigated.

We select the tumour models most similar (biologically, genetically and in drug response) to human cancer. Where the science permits we will use tumours under the skin, as those mice will have fewer health issues than other cancer models (e.g., directly injected into the veins), reducing the suffering of individual mice used for therapeutic studies. Some of the mice that are tumour recipients will be genetically altered to express certain markers for the detection of specific cell types. Others may be genetically altered to modify a gene suspected of being involved in the response (or lack of response) of the tumour to certain therapies.

Most mice in studies will be killed before they show any signs of illness, because the scientific endpoint of the study is reached before the cancer is too advanced, but mice will be killed if they do develop signs of cancer-related illness or drug-related harmful effects, to reduce pain, suffering, distress and lasting harm.

Infected mice may experience clinical signs of infection, such as weight loss, reduced activity and motility, difficulty to breathe, and in accessing water and food. Vaccinated mice will receive multiple treatments which carry a risk of adverse effects. Any animal exceeding or likely to exceed moderate severity or weight loss superior to 15% of the initial weight will be immediately killed by a Schedule 1 method.

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

Rodents are the species with the lowest neurological sensitivity likely to produce data predictive of the effect in man. Also, the majority of animal models of infection, vaccination and cancer have been developed in mice, and there is a longstanding literature on immune responses, cancer biology and pharmacology based on mice, which provides the background to our studies. These models require adult animals, during this stage of life,



the immune system is totally developed and can be challenged with different vaccine platforms. Aged mice may be used to mimic vaccine responses in late stages of life, e.g., old people.

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

We have experience using genetically modified and unmodified mice utilising the immunisation protocols we intend to use under this license. Adverse effects with model proteins – that stimulate the immune system without the need for a pathogen - are rare and are similar to those induced by vaccination in humans. Some inflammation and discomfort may be induced with the use of adjuvants, a vaccine component which helps make the vaccine stronger and more effective, although this should be minimal. The use of genetically modified mice with defects in their immune system may produce an immune deficiency. However, as the mice are kept and bred in the animal unit under specific pathogen- free conditions and do not experience infection, the mice should be unaffected.

When pathogens are used these will be attenuated and the mice will be monitored appropriately. Attenuated pathogens mean that they are altered in a way that makes them less harmful or less able to cause disease, but still able to stimulate the immune system. The body temperature may be taken post-infection as a drop or rise in temperature could be a precursor to clinical signs. If that is the case, this will trigger the provision of wet mash and/or additional warmth via a heat pad or chamber before the signs are seen in the animal. Should the pathogen be used in a mouse strain which we have not used before a pilot study will be carried out under guidance from our animal facilities to establish the most refined protocol.

Our laboratory and animal facilities have experience in producing bone marrow-transplanted mice and strict written protocols will be followed, which may include the administration of antibiotics.

We have been using tumour models and most of the procedures for years already and so the methods are already refined. However, we will scrutinise new guidance as it becomes available and will look to adopt new best practices when advances are published. For procedures we have not used before, we will use pilot studies to develop the protocol and evaluate the best method. As our understanding increases of the timescale for the spreading of tumours (metastasis) in our models, we will refine our endpoints for those studies to avoid mice suffering from the presence of metastases.

For mice receiving modified diet, those specifically susceptible to diabetes will be monitored to detect metabolic alterations or clinical signs of diabetes including excessive urine production and dehydration (rapid weight loss). Urine glucose will be measured if appropriate.



For prolonged oral treatment (daily more than 10 times), we plan to refine this method by guiding the mouse to consume the solutions by the oral route voluntarily rather than administration directly to the stomach.

We will use the guidance documents the NVS and the local AWERB 3Rs Committee have generated, such as the tamoxifen guidance document. Tamoxifen is a medicine that is often used to treat breast cancer in humans. However, in mouse studies, tamoxifen is used differently, mice are treated with tamoxifen to change specific genes in their bodies. This helps us understand how different genes affect the body and how diseases might develop or be treated.

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

We minimise suffering by adhering to best practice guidance, currently the “NCRI Guidelines for the welfare and use of animals in cancer research” by P.Workman, et al., Br. J. Cancer (2010) 102, 1555- 1577.

3Rs resource library. <https://nc3rs.org.uk/> ARRIVE guidelines: <https://arriveguidelines.org/>

NORECOPA's PREPARE guidelines: <https://norecopa.no/prepare> For surgical procedures:

LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section. (E Lilley and M. Berdoy eds.). <http://www.lasa.co.uk/publications/>

For recording and reporting on experiments:

Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLOS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412

Recently published literature in indexed journals of animal experimentation.

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

Via Mailing lists for initiatives from the NC3Rs, information circulated locally by the Named Information Officer and Named Animal Care and Welfare Officers, and information from local annual 3Rs symposia. In addition, one member of our lab sits on the local AWERB 3Rs Committee, so we will have the latest advances in the 3Rs.

Changes to practice would be considered and pilot studies performed to ensure any changes do not compromise the ongoing science.

A retrospective assessment of refinement will be due by 28 February 2030

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. Assessment of novel treatments against acute bacterial infections

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
- 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)

Key words

Bacteria, Infection, Acute, Sepsis

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.

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 overall project aim is to support development of new antimicrobial candidates and novel approaches for the treatment and prevention of acute bacterial infections.



A retrospective assessment of these aims will be due by 6 March 2030

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 lack of new antibiotics and alternative antimicrobials being developed has led to a surge of resistant bacterial strains, particularly amongst common bacteria leading to an increase in life threatening infections. There is an urgent need to develop and assess efficacy of novel antimicrobials and improve our understanding of suitable treatment regimens against different bacterial strains and routes of infection.

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

The data generated under this project will provide information on lead compounds' efficacy:

New information on beneficial effects when compared to a placebo control e.g., saline.
New information on beneficial effects when compared to a reference drug.

All data generated will be analysed prior to being recorded in our archives (electronic files and/or hard copies). All data from a given study will be compiled into a report comprising: (i) a short introduction including the aim(s) of the study, (ii) a materials and methods section including an outline of the experimental protocol, a summary of the experimental groups and a description of each readout, (iii) a results section including graphical representations of the analysed data, statistical analyses and interpretations, (iv) a conclusion section with a summary of all findings and (v) a raw data section. The study outcome, whether judged 'positive' (e.g., beneficial effect of the lead compound when compared to a reference drug) or 'negative' (e.g., no beneficial effect of the lead compound when compared to a vehicle and/or no greater beneficial effect of the lead compound when compared to a reference drug and/or observed adverse events) will be reported.

Some data may be used to generate scientific literature. These data are made available to the scientific community (i) during scientific conferences attended by us or our clients, (ii) upon request through our website or (iii) through targeted email campaigns and social media.

Some data may be used for meta-analysis to refine experimental designs and experimental methods and to reduce the number of animals used.

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

The initial data generated will be informative and help the Sponsor to decide the next steps



i.e., further improvements to the test compound to improve effect against bacterial infection, assess alternative dosing regimen i.e., increase dose and/or frequency of administration. If data is promising the Sponsor may want to carry out additional efficacy studies or move on to pre-clinical toxicology, to assess safety of the drug. Alternatively, the data may lead to test compound being rejected due to the lack of a positive effect against the infection or due to poor safety data indicating toxic effect.

By contributing to the development of new candidate drugs for the treatment of bacterial infections, our project will benefit treatment of patients with acute bacterial infections, limiting onset of sepsis and improving survival. Typically, we would test 20 compounds every year in models of acute bacterial infection.

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

The outputs of this work will help guide next steps. Where possible data will be shared at conferences, on social media and in publications. The data will aid future studies, add to our experience to advise clients, and provide information to other scientists.

Species and numbers of animals expected to be used.

- Mice: 3000
- Rats: 250

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.

Many different strains of both rats and mice have been established which provide models of a range of human diseases. Due to their size, ease of reproduction and handling, many aspects of the immune response, leading to disease caused by inflammation and autoimmunity, have been well characterized. The genetics of these strains is well understood, aiding our understanding of disease development and potential interventions. Thus, mice and rats are, for many diseases, the most appropriate species for early assessment of potential novel therapies.

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

All animals will have temperature reading transponders (microchips) inserted prior to infection, under the skin, between the shoulder blades. Some may be immunocompromised following administration of specific compounds several days prior to infection. Thereafter, animals will be infected with bacteria via an appropriate route. Treatments or controls will also be administered on by an appropriate route on several occasions following the Laboratory Animals Science Association (LASA) guidelines for volumes and frequencies. These animals will then be monitored regularly for onset and progression of clinical disease and body temperature changes.

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



Animals may experience discomfort at the administration site, but may also experience clinical signs, including coat condition, abnormal posture, reduced mobility, abnormal breathing (with termination if breathing becomes laboured), presence of ocular discharge/squinting. Furthermore, despite regular checks a small percentage may be found dead as a result of sudden onset of sepsis leading to organ failure.

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)?

80% Moderate, 20% Severe

What will happen to animals used in this project?

- Killed

A retrospective assessment of these predicted harms will be due by 6 March 2030

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?

Infection and the immune system's response to the presence of bacteria, toxins and other antigenic components, involves multiple systems, multiple organs and multiple cell types. The complexity of the response cannot be fully reproduced in vitro.

The interaction between host and pathogen is an important driver of expression of different virulence factors and emergence of resistance. While some individual aspects of these processes can be modelled in vitro, the complexity of the interactions between multiple body systems that is critical to understanding these diseases and in defining treatments cannot.

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

In vitro studies:

Potential antimicrobial compounds are initially assessed for efficacy in in vitro systems, typically assessment of minimum inhibitory concentration (MIC), but other assays include minimum bactericidal activity and time-kill assays which help determine optimum dosing regimens. Further, we can carry out cell-based assays where applicable to determine inhibitory effect on bacterial internalisation and/or killing.

Additional in vitro models are currently being assessed including organ on a chip to help



model chronic lung infection as well as organ transplants e.g., skin and lung explants.

We have also validated the use of *Galleria mellonella* (wax moth larvae), for early assessment of antimicrobials against several strains of bacteria. This work was initially in conjunction with Biosystems Technology and now with Exeter University who provide truLarv larvae which are bred free of hormones and antibiotics, genome sequenced and surface sterilised, which ensures reproducible data. These larvae have an innate immune response similar to that in mammalian systems so provides a useful intermediate between *in vitro* and animal models of infection.

This model also provides information on tolerability as well as PK and PD data for novel compounds and thus helps to optimise dosing regimen.

Why were they not suitable?

Alternatives to animal models are a great addition in the assessment of test compounds and can help to confirm mode of action. Most test compounds have already been assessed *in vitro* by either ourselves or the sponsor. However, due to the additional complexity of the human body and interactions of immune cells and pathways, it is not possible to replicate this fully *in vitro*, and *in vivo* studies are unfortunately still required to fully understand and show efficacy.

A retrospective assessment of replacement will be due by 6 March 2030

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 is estimated from previous animal returns and expected demand in coming years.

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

Use of *in vitro* assays, including potential 2 and 3-dimensional set-ups, will minimise the number of animals required for initial assessment of potential novel compounds.

In addition, utilising the National centre for implementation of replacement, reduction and refinement of animal use in research (NC3R's) guidelines and the experimental design assistant can help to balance group sizes. Any naïve, untreated or vehicle treated animals will be kept to a minimum while still allowing meaningful data to be assessed for statistical significance. Objective of the study e.g., a dose finding or efficacy as well as the incidence



of disease and variability of the model will impact group sizes. Assessment of previous data or review of the literature will also help guide the group sizes as well as the number of control groups required. While we work to GLP-like standards, however, we do not have to comply to additional regulatory guidelines.

In addition, we make good use of new technologies such as the in vivo imaging systems (IVIS) which enables the level of infections to be monitored during the course of infection, without the need to terminate animals at different time points, thus reducing the number of animals required for assessment of bacterial load. We have a good selection of bioluminescent strains which have been validated in several models of infection.

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

Pilot studies are undertaken to ensure suitable bacterial strains are used, at an appropriate inoculum, that provides the expected incidence of disease with minimal variability.

Assessment of previous data or review of the literature will also help guide, choice of strain, inoculum, the group sizes as well as ensuring appropriate control groups are included. Additional samples may be taken at when animals are killed at the end of the study to provide control data for future studies or for optional, additional readouts.

A retrospective assessment of reduction will be due by 6 March 2030

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 animals to be used under this license are limited to rodents (mice or rats). Mice are the species that will be used by default. Rats will only be used when the infection cannot be effectively modelled in mice, when the lead compound has poor bioavailability in mice or when the blood volumes or tissue quantity required for multiple assays are not compatible with sampling from mice.

Bacterial strains not previously used, or that have not been used recently i.e., in the past year, will be assessed for virulence in a pilot study to ensure the optimum disease course using a small number of animals. Only when infection models are well defined in terms of inoculum and humane endpoints will potential compounds be assessed. Each model of infection has clearly defined endpoints to ensure that they are not kept alive beyond the



point from which they may recover. The severity of these models will vary depending on the model of interest and strain used. Many bacterial infections in humans cause severe disease and are life-threatening, therefore it is important to replicate this situation *in vivo* to determine the potential efficacy of a novel antimicrobial or immunomodulatory compound.

However, in deciding on a suitable model for testing a novel treatment or therapy, the mode of action and target will be considered together with the most appropriate model that will cause least harm to achieve the scientific objectives. Where bacterial infection studies are undertaken, the bacteria and mouse strain of choice that results in sufficient disease to ensure successful outcome while causing least harm will be used. The least invasive route of administration will be used, and any surgery will only be undertaken if justified. Where disease severity is high, determination of humane endpoints and frequency of monitoring is essential to ensure any suffering is kept to a minimum.

We ensure adverse events are identified early by (i) starting to monitor the animals immediately after disease-induction and prior to the expected onset of clinical signs, (ii) monitoring the animals frequently and (iii) increasing the monitoring regimen at times of peak disease. Animals are monitored for change in body temperature using subcutaneously implanted microchips and for disease-specific clinical signs which include changes in appearance (posture, piloerection), respiratory rate and mobility.

All protocols have humane endpoints relative to the model which will be enforced if clinical signs reach severity limits. Heat mats may be used where a sudden drop in body temperature is observed due to sudden cytokine release following infection, i.e., a cytokine storm.

Where bioluminescent bacteria are able to be used we can track the progression of the infection throughout the course of disease, without the requirement for satellite groups of animals to be killed at different timepoints, thus reducing the number of animals required.

Genetically modified animals may also be used where a particular target or pathway is relevant to the disease profile or treatment.

When collecting excreta we have specific caging that houses up to 6 mice at a time, but allow them to smell and hear one another, so that they are not completely isolated.

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

Most of our studies will be done in mice, which are the lowest species that develop these diseases in the same way as humans. Rats are occasionally used when the disease cannot be modelled in mice, if the test compound does not work appropriately in mice, or if a larger animal is needed for sampling requirements.

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

Animal suffering will be limited by ensuring that the methods used cause the least amount of harm to the animals. The mildest bacterial dose will be used to induce consistent, reproducible levels of disease and studies will be kept to as short a duration as possible.

Animals are monitored frequently for signs of discomfort, and frequency increased as animals show increasing adverse effects as a consequence of infection. Animals will be



treated to control adverse effects or humanely killed once they reach agreed endpoints or if they develop signs of severe suffering.

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

We follow the recommended Best Practice guidelines of Turner et al. 2011 and Diehl 2001 and LASA, as recommended by the Home Office. We keep up to date by receiving regular updates from the Home Office and LASA, as well as information provided by the Institute of Animal Technology (IAT), following the literature, internal forums and advice from our dedicated veterinary surgeon.

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

We keep up to date with NC3Rs guidelines by online updates as well as attending Institute of Animal technology (IAT) Congress and Laboratory Animal Science Association (LASA) conferences, HOLTIF forum and workshops.

We also check the literature to ensure the models we are using are the most appropriate and include additional validation of models where improvements can be made.

A retrospective assessment of refinement will be due by 6 March 2030

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. Assessment of novel treatments against chronic bacterial infections

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

Bacteria, Infection, Chronic, Antimicrobial

Animal types	Life stages
Mice	adult, aged
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 overall project aim is to support development of new antimicrobial candidates and novel approaches for the treatment and prevention of chronic bacterial infections.

A retrospective assessment of these aims will be due by 10 March 2030

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?

Chronic infections affect millions of people worldwide causing pain, impaired function, and diminished quality of life. By contributing to the development of new anti-microbial drugs and novel therapies, our project will benefit the patients, improving their quality of life and reducing suffering. By providing high quality services and scientific expertise, we can make the testing of such drugs more cost effective, more informative and reduce the need for companies to set up the models in house.

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

The data generated under this project will provide information on lead compounds' efficacy:

- (1) New information on beneficial effects when compared to a placebo control eg., saline
- (2) New information on beneficial effects when compared to a reference drug

All data generated will be analysed prior to being recorded in our archives (electronic files and/or hard copies). All data from a given study will be compiled into a report comprising: (i) a short introduction including the aim(s) of the study, (ii) a materials and methods section including an outline of the experimental protocol, a summary of the experimental groups and a description of each readout, (iii) a results section including graphical representations of the analysed data, statistical analyses and interpretations, (iv) a conclusion section with a summary of all findings and (v) a raw data section. The study outcome, whether judged 'positive' (e.g., beneficial effect of the lead compound when compared to a reference drug) or 'negative' (e.g., no beneficial effect of the lead compound when compared to a vehicle and/or no greater beneficial effect of the lead compound when compared to a reference drug and/or observed adverse events) will be reported.



Some data may be used to generate scientific literature. These data are made available to the scientific community (i) during scientific conferences attended by us or our clients, (ii) upon request through our website or (iii) through targeted email campaigns and social media.

Some data may be used for meta-analysis in order to refine experimental designs and experimental methods and to reduce the number of animals used.

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

The initial data generated will be informative and help the Sponsor to decide the next steps i.e., further improvements to the test compound to improve effect against bacterial infection, assess alternative dosing regimen i.e., increase dose and/or frequency of administration. If data is promising the Sponsor may want to carry out additional efficacy studies or move on to pre-clinical toxicology, to assess safety of the drug. Alternatively, the data may lead to test compound being rejected due to the lack of a positive effect against the infection or due to poor safety data indicating toxic effect.

By contributing to the development of new candidate drugs for the treatment of chronic infectious diseases, our project will benefit patients improving their quality of life and helping to reduce or prevent the effects of chronic bacterial infections. Typically, we would test 20 compounds every year in models of chronic bacterial infection.

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

The outputs of this work will help guide next steps. Where possible data will be shared at conferences, social media and in publications. The data will aid future studies, add to our experience to advise clients, and provide information to other scientists.

Species and numbers of animals expected to be used

- Mice: 9000
- Rats: 1750

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.

Many different strains of both rats and mice have been established which provide models of a range of human diseases. Due to their size, ease of reproduction and handling, many aspects of the immune response, leading to disease caused by inflammation and autoimmunity, have been well characterized. The genetics of these strains is well understood, aiding our understanding of disease development and potential interventions.



Thus, mice and rats, are, for many diseases, the most appropriate species for early assessment of potential novel therapies.

Adult animals are generally the most appropriate to use, however, occasionally we may need to use neonates, new-borns, juveniles, or aged animals to better assess the impact of a treatment prior to, or at time of disease onset, particularly where development of disease is spontaneous and may occur early or later in life. Further, the immune response can be affected by age, so use of aged animals in some models may also be required.

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

Mice will be preferred for most studies, but rats will be used when the lead compound has poor bioavailability in mice, the rat provides a more clinically relevant model and endpoints, or a larger volume of sampling for ex vivo analysis is required.

Typically, animals will be infected with a suitable bacteria by the appropriate route which results in infection of different organs. For example, infection of the lung which may lead to pneumonia will be achieved by inoculation of bacteria via the nasal passages or the trachea. Wound infections will involve injection of bacteria into the skin or administration on to the surface of an area of the skin following abrasion. Urinary tract infections are established by administration of bacteria via urethra, using a canulae. Infected animals will be monitored for signs of clinical disease, bodyweight loss and changes in temperature.

In some models, animals may be injected with an immunosuppressant drug to aid infection, similar to immunocompromised patients that are more susceptible to bacterial infection.

Therapies may be given prior to and/or following infection by a variety of routes. For example, the testing of vaccines requires their administration prior to the establishment of infection, whereas the administration of novel anti-microbials would normally be post-infection to replicate treatment of patients with chronic bacterial infection. The most appropriate route and regimen will be selected on the basis of an understanding of the activity of the therapy being given. Blood samples, faeces and/or urine may be collected at various timepoints for analysis of drug and/or bacteria.

Some protocols contain more unusual, invasive, or uncommon methods of administration and/or monitoring disease. These are explained and justified in more detail below:

Osmotic mini-pumps: These are small infusion pumps for unrestrained dosing of compounds into laboratory animals. They are a convenient and reliable alternative to chronic or repeated injections and



ensure a constant compound level for maximised therapeutic efficacy and reduced adverse effects. They are subcutaneously implanted into the animal, usually between the shoulder-blades in a quick and minimally invasive procedure. Analgesics are given in-line with NVS advice.

In vivo imaging: Animals may be imaged, under anaesthesia, to visualise bioluminescence or fluorescence markers associated with the bacteria or drug. This enables us to monitor disease progression and any subsequent efficacy of compounds, non-invasively, without the need to terminate animals at various timepoints. Imaging sessions generally last no longer than 5 minutes with animals anaesthetised throughout and will typically be limited to 3-4 times a week. Where aged animals are used, animals will have additional monitoring of age-related conditions to ensure animals are not suffering due to complications associated with age.

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

When inducing infection, we expect to see some clinical signs relating to the disease. The severity, duration and observations will depend on the model, route of administration and bacterial strain used. Chronic infections typically do not cause clinical signs leading to mortality but mild to moderate observations such as piloerection, abnormal posture, reduced activity, and changes to breathing patterns may be observed. Body weight loss and abdominal swelling may also be observed.

The caecal ligation and puncture (CLP) model involves surgery leading to some discomfort and potential lethargy associated with the procedure and effect of anaesthesia. In a small proportion of the animals, approximately 5%, infection may lead to sepsis and organ failure causing animals to show reduced mobility including loss of limb co-ordination. Measures are taken to limit harms such as frequent monitoring of disease-specific clinical signs and non-specific clinical signs for early identification of adverse events. Clinical signs are not expected for more than 72 hours during which the animals will be monitored regularly and terminated upon reaching endpoints outlined below. At the end of an experiment, all animals will be humanely killed to enable further ex vivo testing of samples.

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)?

Both rats and mice are expected to experience mild to severe severity during the course of infection. A small percentage, <5%, may experience severe adverse effects as a consequence of the infection, while approximately 75% are expected to show moderate clinical signs and approximately 20% will be mild.



What will happen to animals used in this project?

- Killed

A retrospective assessment of these predicted harms will be due by 10 March 2030

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?

Infection and the immune system's response to the presence of bacteria, toxins and other antigenic components, involves multiple systems, multiple organs and multiple cell types. The complexity of the response cannot be fully reproduced in vitro.

The interaction between host and pathogen is an important driver of expression of different virulence factors and emergence of resistance. While some individual aspects of these processes can be modelled in vitro, the complexity of the interactions between multiple body systems that is critical to understanding these diseases and in defining treatments cannot.

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

In vitro studies

Potential antimicrobial compounds are initially assessed for efficacy in in vitro systems, typically assessment of minimum inhibitory concentration (MIC), but other assays include minimum bacteriocidal activity and time-kill assays which help determine optimum dosing regimens. Further, we can carry out cell-based assays where applicable to determine inhibitory effect on bacterial internalisation and/or killing.

Additional in vitro models are currently being assessed including organ on a chip to help model chronic lung infection as well as organ transplants e.g., skin and lung explants.

We have also validated the use of *Galleria mellonella* (wax moth larvae), for early assessment of antimicrobials against several strains of bacteria. This work was initially in conjunction with Biosystems Technology and now with Exeter University who provide truLarv larvae which are bred free of hormones and antibiotics, genome sequenced and surface sterilised, which ensures reproducible data. These larvae have an innate immune response similar to that in mammalian systems so provides a useful intermediate between in vitro and animal models of infection.



This model also provides information on tolerability as well as PK and PD data for novel compounds and thus helps to optimise dosing regimen.

Why were they not suitable?

Alternatives to animal models are a great addition in the assessment of test compounds and can help to confirm mode of action. Most test compounds have already been assessed in vitro by either ourselves, or the sponsor. However, due to the additional complexity of the human body and interactions of immune cells and pathways, it is not possible to replicate this fully in vitro, and in vivo studies are unfortunately still required to fully understand and show efficacy.

A retrospective assessment of replacement will be due by 10 March 2030

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 is estimated from previous animal returns and expected demand in coming years.

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

Use of in vitro assays, including potential 2 and 3-dimensional set-ups, will minimise the number of animals required for initial assessment of potential novel compounds. In addition, utilising the National centre for implementation of replacement, reduction and refinement of animal use in research (NC3R's) guidelines and the experimental design assistant can help to balance group sizes. Any naïve, untreated or vehicle treated animals will be kept to a minimum while still allowing meaningful data to be assessed for statistical significance. Objective of the study e.g., a dose finding or efficacy as well as the incidence of disease and variability of the model will impact group sizes. Assessment of previous data or review of the literature will also help guide the group sizes as well as the number of control groups required. While we work to GLP-like standards, however, we do not have to comply to additional regulatory guidelines.



In addition, we make good use of new technologies such as the in vivo imaging systems (IVIS) which enables infections to be monitored 'in-life', thus reducing the number of animals required for assessment of bacterial load. We have a good selection of bioluminescent strains which have been validated in several models of infection.

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

Pilot studies are undertaken to ensure suitable bacterial strains are used, at an appropriate inoculum, that provides the expected incidence of disease with minimal variability. Assessment of previous data or review of the literature will also help guide, choice of strain, inoculum, the group sizes as well as ensuring appropriate control groups are included. Additional samples may be taken at termination to provide control data for future studies or for optional, additional readouts.

A retrospective assessment of reduction will be due by 10 March 2030

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 animals to be used under this license are limited to rodents (mice or rats). Mice are the species that will be used by default. Rats will only be used when the infection cannot be effectively modelled in mice, when the lead compound has poor bioavailability in mice or when the blood volumes or tissue quantity required for multiple assays are not compatible with sampling from mice.

Efficacy models listed in this licence are validated models, although not with all bacterial strains. The models of infection include respiratory, wound, peritonitis and urinary tract infection (UTI), have been published in peer-reviewed journals by academic or industrial groups. The models have been used to test the efficacy of anti-microbial drugs currently used in the clinic. Some models have been used here for over ten years; however, we still continually check for newer models and ensure that best practice is followed. We evaluate



techniques continually to determine whether there are refinements that can be made or if scientific outputs can be met using shorter or less severe models.

Bacterial strains not previously used, or have not been used recently i.e., in the past year, will be assessed for virulence to ensure the optimum disease course using a small number of animals. Only when infection models are well defined in terms of dose and humane endpoints will potential compounds be assessed. Each model of infection has clearly defined endpoints to ensure that they are not kept alive beyond the point from which they may recover. The severity of these models will vary depending on the model of interest and strain used. Many infectious models in humans cause severe disease and are life threatening, it is therefore important to replicate this situation in vivo to determine the potential efficacy of a novel antimicrobial. However, in deciding on a suitable model for testing a novel treatment or therapy, the mode of action will be considered together with the most appropriate model that will cause least harm to achieve the scientific objectives. Where bacterial infection studies are undertaken, the bacteria and mouse strain of choice that results in sufficient disease to ensure successful outcome while causing least harm will be used. Where disease severity is high, determination of humane endpoints is essential to ensure any suffering is kept to a minimum.

We ensure adverse events are identified early by (i) starting to monitor the animals immediately after disease-induction and prior to the expected onset of clinical signs, (ii) monitoring the animals frequently and (iii) increasing the monitoring regimen at times of peak disease. Animals are monitored for

disease-specific signs which include changes in appearance (posture, piloerection), respiratory rate and mobility. All protocols have humane endpoints relative to the model which will be enforced if clinical signs reach severity limits.

If required, animals are provided with food pellets, and/or a bowl of mashed food (water soaked), on the cage floor where disease induction may impact mobility or desire to eat.

Where minor surgical procedures are performed, for example, insertion of minipumps, pins or scaffolds analgesics will be given, in line with veterinary guidance.

During the course of this project licence, we will continue to evaluate the use of mouse grimace scores (MGS) as described by Langford et al (2010), in line with the NC3Rs, as an indication of signs of pain. Such scores may provide an early indication of the onset of clinical disease, leading to increased monitoring. If the data gives reliable assessment of disease onset, this may be incorporated into future license applications or amendments and help towards further refinement of models.



Score	Eyes tighten ing	Ear positioni ng	Nose bulgin g	Cheek bulgin g	Whisker positionin g	Piloerection	Gait	Activity
0	0%	Normal	None	None	Normal	None	Normal	Normal
1	0-50%	Mild	Mild	Mild	Mild	Mild/Intermit tentMild	Mild	Mild
2	>50%	Marked	Mark ed	Marke d	Marked	Marked	Marked	Marked

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

Most of our studies will be done in mice, which are the lowest species that develop these diseases in the same way as humans. Rats are occasionally used when the disease cannot be modelled in mice, if the test compound does not work appropriately in mice, or if a larger animal is needed for sampling requirements.

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

Animal suffering will be limited by ensuring that the methods used cause the least amount of harm to the animals. The mildest bacterial dose will be used to induce consistent, reproducible levels of disease and studies will be kept to as short a duration 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.

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

We follow the recommended Best Practice guidelines of Turner et al. 2011 and Diehl 2001 and LASA, as recommended by the Home Office. We keep up to date by receiving regular updates from the Home Office and LASA, as well as information provided by the Institute of Animal Technology (IAT), following the literature, internal forums and advice from our dedicated veterinary surgeon.

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

We keep up to date with NC3Rs guidelines by online updates as well as attending Institute of Animal technology (IAT) Congress and Laboratory Animal Science Association (LASA) conferences, HOLTIF forum and workshops.

We also check the literature to ensure the models we are using are the most appropriate and include additional validation of models where improvement can be made.



A retrospective assessment of refinement will be due by 10 March 2030

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. ADME and Pharmacokinetic Studies

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

Metabolism, Pharmacokinetics, Human Health, Safety

Animal types	Life stages
Mice	adult
Rats	adult, neonate, pregnant
Guinea pigs	adult
Rabbits	adult, pregnant
Animal types	Life stages
Beagles	adult
Pigs	adult
Minipigs	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 aim of studies under this license in the development phase is to meet international



regulatory requirements to provide information on the absorption, distribution, metabolism and excretion (ADME) of drugs in the species being used to generate safety data. This information is used to ensure that the safety assessment data obtained in the toxicology studies fully covers human exposure of the drug and its metabolites. ADME programmes are generally conducted using radiolabelled test compounds to provide a simple means of tracking the administered dose within the body.

Studies performed under the license in the discovery/research phase are designed to assist sponsors select development candidates based on pharmacokinetic (PK) - absorption, distribution, metabolism and excretion properties.

A retrospective assessment of these aims will be due by 16 March 2030

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?

It is important to undertake this work for the identification and development of safe, effective drugs.

Discovery/Research studies enable the pharmaceutical industry to efficiently select suitable development candidates and prevent compounds with “poor” PK properties entering the development process (historically one of the main reasons for drug candidate failure).

Absorption, distribution, metabolism and excretion information forms a key part of the safety assessment process. The testing enables the pharmaceutical industry to meet regulatory requirements for drugs allowing them to progress through clinical development and meet international regulatory requirements. Patients benefit once the developed products reach the market.

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

The outputs of this project will be new information on the safety and ADME/PK properties of potential new treatments for diseases in man. This information will then be used in setting appropriate dose levels in further studies, such as clinical studies in man or toxicology studies.

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

Development work beneficiaries are patients and the pharmaceutical/biotechnology industry. The testing performed facilitates the pharmaceutical industry in meeting regulatory requirements for drugs allowing them to safely and efficiently progress through clinical development and meet international registration requirements.



Patients benefit once development products reach the market but this is inevitably a long term benefit.

Looking at clinical trials success rates and development times within the next 5 years at least three new treatments would be expected to be a registered commercial product significantly improving human health. Over the period of the last license we can see the following products going into clinical trials that we contributed to developing through the careful use of animals:

Key Phase 1/1b efficacy data support an ATR inhibitor is active across tumour types Molecular responses and reduction in tumour size observed in multiple cancer types including those harboring molecular alterations or biology consistent with high replication stress. Durable confirmed responses in all enrolled patients with high grade endometrial cancer.

Potent tumour-specific DNA damage detected by selective increases in γ H2Ax in circulating tumour cells but not normal cells in DDR deficient tumours predicted through the client DcoDeR platform.

Key Phase 1/1b safety and PK data demonstrates compound is well tolerated and predictable. Safe and well tolerated at intermittent and continuous doses.

Predictable, manageable and reversible on target anaemia. Dose proportional increases in exposure with rapid absorption and relatively rapid elimination and low variability.

We supported this programme with PK studies looking at formulation optimisation and a full ADME radiolabel package.

Treatment of hereditary angioedema (HAE) is an autosomal dominant disease caused by either a lack of C1-inhibitor protein or dysfunctional C1-inhibitor protein.

HAE manifests with symptoms related to angioedema of the upper airway, skin, and/ or gastrointestinal tract. Compound successfully reached all endpoints in Phase 3 clinical study with safety better than placebo. NDA submission to the FDA expected first half of 2024.

We supported this work by providing PK studies on the compound.

Compound was approved by FDA in December 2023. This is a treatment for ATTR polyneuropathy, a systemic, progressive and fatal neurological disease.

We supported this work with a full ADME radiolabeled package.

We ran two subcutaneous ADME studies in rats with this GalNAc conjugated oligonucleotide. The first involved tritium labelling of the GalNAc linker moiety, which allowed us to provide Ionis with detailed information on the metabolism and biodistribution of GalNAc portion of the molecule. In the second study the oligonucleotide was tritium labelled, which allowed us to follow the distribution and metabolism of the oligonucleotide portion of the molecule in more detail.

Inevitably the drug development process is a long one and as a CRO we do not have



visibility on how most of the products we work on progress but in the long term this work plays an important role in the development of novel medicines.

The research studies undertaken principally benefit the pharmaceutical industry in helping them efficiently select suitable development candidates this is in general a short to medium term benefit. In addition the research services aid optimisation of the formulation, dose and route of administration used in safety assessment and clinical practice.

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

We perform Research and Development studies on new techniques and offerings, through internal Research and Development studies, then presents these through talks and posters at various conferences. Examples of these are the collection of blood samples through the use of an indwelling catheter reducing the number of needle sticks an animal receives during the course of a study.

Presenting new information on Quantitative Whole Body Autoradiography data as this significantly reduces the number of animals required compared to the historical approach as only one animal per time point is required compared to $n=3$ or more on traditional studies.

We have a continual refinement in sample collection techniques specifically collecting extremely small samples of blood, typically 20microlitres from smaller rodent species such as mice or rats.

We also share our knowledge in performing procedures as personal licence holders gain very good procedural skills when repeatedly performing a procedure. An example of this is the serial bleeding of pigs from the jugular vein without the need for surgical cannulation. This improves the welfare for the pigs as they do not have to undergo surgery and reduces the number of animals used as the pigs can be used for longer. Implanting a jugular cannula in a pig has a limited life span due to the pigs growth and being able to repeatedly serially bleed a pig from the jugular, with minimal impact on the pig apart from the moment of pain as the needle is inserted, is both a refinement and reduction.

Species and numbers of animals expected to be used

- Beagles: 130
- Pigs: 500
- Minipigs: 100
- Mice: 6550
- Rats: 11900
- Guinea pigs: 170
- Rabbits: 190

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 work to regulatory guidelines, where appropriate, and as such the species of animals



and their life stage are stipulated by the regulatory authorities.

We only use adult animals as this is the accepted regulatory standard for these study types.

ADME/QWBA/PK studies in rodents and lagomorphs (Incl. surgical and non-conventional routes of administration); generally rats and mice will be used for studies of this type and on rare occasions rabbits and guinea pigs will be used.

Rodents would be the first choice as they are the least sentient animals in this license. Rodents are a commonly used animal in these study types.

ADME/PK studies in dogs, pigs and mini pigs

Adult dogs are a commonly used species in pre-clinical research due to their similar physical and cognitive characteristics to man and our experience based on the behaviour of compounds in them as a safety assessment model over many years.

Registration of pharmaceuticals for human use generally requires the inclusion of a non-rodent species in the safety assessment process, performed mainly in the dog but can also be performed in the pig or mini pig. Where possible we explore the possibility of using pigs instead of dogs although the species selection is mainly driven by previously conducted in-vitro and in-vivo work. It is also governed by the species selected for toxicological and efficacy studies.

The species used, whether it be adult dogs or pigs, is dictated by the species most applicable to the candidate under investigation, such as the species being used in toxicity studies or the most suitable species accessed by in-vitro comparison metabolism testing.

Tissue distribution studies (QWBA) in rodents and lagomorphs

Generally adult rats and mice are used but occasionally guinea pigs or rabbits may be selected. Rats or mice are the preferred choice due to the limitations in sectioning larger species and the historical data approach in drug development of using rats for this study type.

Collection of bile

This is performed in adult rats only. Rats are used as this species has historically been the choice for this study type. Rats provide enough bile sample for analysis and are able to provide a time course of bile production meaning that a whole time course can be analysed from n=3 rats.

Collection of milk

For milk studies adult female rats are used. They are selected as they are the least sentient animals we can use on this study type whilst still collecting enough milk for analysis.

Placental transfer

Only adult rats and rabbits will be used under this Protocol. Primarily rats will be used as they are least sentient species and the fact that pregnant rats can easily be bought from a designated breeder.

Blood collection by controlled exsanguination

Generally rats and mice which are fully mature will be used for this type of project. This is



dictated by the majority of pre clinical studies being performed in these species and as such there is more demand for control biological sample from these species for in-vitro and bio analytical areas of work.

QWBA and PK in tumour bearing rodents

Adult Mice or rats inoculated with subcutaneous tumour cells may be used for candidate PK assessment or QWBA. QWBA studies use adult animals as these are generally used in toxicology and clinical studies- so it is important to quantify this study type in adults. PK studies are conducted in tumour bearing mice to assess the effect of a tumour bearing mice on the candidate PK characteristics. Rats and mice are the commonly used least sentient species for this study type. Oncology studies generally use rats and mice only due to their short gestation cycle and historical breeding programmes with immuno compromised and genetically altered mice and rats.

Conventional PK studies in rodents and lagomorphs

Adult Rats and mice are the species most used most conducting this study type. This is due to their ease of buying from a designated breeder and ease to work with. They are the least sentient species we can use. Guinea pigs and rabbits have many biological similarities to humans, beyond the simple fact that they are mammals, which make them useful in many fields of research. Guinea pigs are widely used in medical research, particularly in the study of respiratory, nervous and immune system areas. To this end both these species are occasionally used by us to support work of this type. Both guinea pigs and rabbits are very docile and non-aggressive and hence easy to handle and observe. They are widely bred and very economical compared with the expense of larger animals and have short vital cycles (gestation, lactation, and puberty).

To achieve the scientific objectives of determining compound concentrations in biological samples initially rodents or lagomorphs will be used then if the data produced highlights a drug candidate with good PK properties a further study may be performed in higher order species such as dog or pig.

Implant development in the anaesthetised pig using a non-recovery model

Only mature pigs will be used under this Protocol. This is due to pigs having a skin structure very similar to humans so the most appropriate species to use with this type of work.

First pass metabolism PK studies using Hepatic Portal Vein cannulated rats

Only mature rats will be used under this Protocol. The rat is being used as it is the only species we can procure from a designated breeder to perform this study type.

The rat can also provide enough blood, based on bodyweight, to meet the objectives of the study.

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

ADME/QWBA/PK studies in rodents and lagomorphs (Incl. surgical and non-conventional routes of administration).

For rodents and lagomorphs the typical severity experience, accounting for ~80% of the animals under this Protocol, will undergo administration of substances via specified routes (at the specified frequencies and maximum durations) followed by blood sampling at the specified volumes and frequencies then killed. For rodents and lagomorphs, the most



severe experience accounting for ~20% of the animals under this Protocol is undergoing surgery to cannulate the intestine or implant of vascular cannula. Then administration by non-conventional route, confinement in metabolic cage for up to a maximum of 28 days, blood sampling within the specified maximum frequencies/volumes then killed.

ADME/PK studies in dogs, pigs and mini pigs. For dogs and pigs the typical severity experience, accounting for ~90% of the animals under this Protocol, will undergo administration of substances via specified routes (at the specified frequencies and maximum durations) followed by blood sampling at the specified volumes and frequencies. The most severe experience, accounting for ~10% of the animals under this Protocol require confinement in a metabolic cage for up to a maximum of 15 days and blood sampling within the specified maximum frequencies and volumes.

Animals that have been kept alive and maintained under the supervision of the NVS may be re-used in this protocol. Each case requires approval from a veterinary surgeon, with knowledge of the lifetime experience of the animals, of fitness of animals to remain alive at the conclusion of the previous study on the basis that: (a) the animals are not suffering and are not likely to suffer adverse effects as a result of the regulated procedures; and (b) that their general state of health and well-being is likely to have been fully restored following the completion of the regulated procedures. Animals that have suffered no more than mildly or moderately during the course of procedures and which are not suffering or likely to suffer as a result may be kept alive in accordance with Standard Condition 11.A standard PK study will generally last up to 24 hours post dose but a radiolabelled study may generally last for 7 to 15 days.

Tissue distribution studies (QWBA) in rodents and lagomorphs (conventional dosing). The typical severity experience, accounting for ~80% of the animals under this Protocol, will undergo administration of substances via specified routes (at the specified frequencies and maximum duration's) followed by blood sampling at the specified volumes and frequencies then killed. The most severe experience, accounting for ~20% of the animals under this Protocol, will undergo administration by conventional routes, confinement in metabolic cage for up to a maximum of 28 days, blood sampling within the specified maximum frequencies and volumes then sacrifice by rapid submersion in a cold fixative (hexane/cardice) ensuring instant death, whilst under terminal anaesthesia.

Biliary excretion and enterohepatic recirculation in rat

This study type involves the placing of surgically prepared animals into individual metabowls, with a jacket, harness and tether attached, to allow the continuous collection of bile samples from the freely moving rat. This procedure is at present the only option available to collect serial bile samples using the least numbers of animals. These surgically prepared animals are only allowed to be kept in metabowls for up to 4 days post dose, with special justification sought to collect beyond 72 hours post dose. Following completion of the sample collections each animal is killed.

Milk secretion

The typical severity experience under this Protocol will undergo administration of substances via specified routes (at the specified frequencies and maximum durations) followed by milk and/or blood sampling at the specified volumes and frequencies then killed. Rats are the only species allowable under this protocol. Following dosing rats will have have milk and/or blood samples collecting post dose.

Placental transfer. The typical severity experience, accounting for all the animals under this Protocol, will undergo administration of substances via specified routes (at the



specified frequencies and maximum durations) followed by blood sampling at the specified volumes and frequencies then killed. A study of this nature will generally last 24 hours.

Blood collection by controlled exsanguination

This is a non-recovery Protocol with no adverse effects expected as no treatments are being administered and blood collection is performed under terminal anaesthesia. Rats, mice, guinea pigs and rabbits may be used under this protocol.

PK and QWBA in tumour bearing rodents. The typical severity experience for animals under this Protocol, will undergo a single subcutaneous inoculation of tumour cells in either the flank or mammary gland. The growing and monitoring of tumour sizes followed by administration of substances via specified routes (at the specified frequencies and maximum durations) then killed for QWBA assessment. We rarely perform PK blood sampling studies in tumour bearing rodents. Studies of this type will generally last for up to 14 days.

Conventional PK studies in rodents and lagomorphs

Conventional PK studies in rodents and lagomorphs. The typical severity experience for animals under this Protocol, will undergo administration of substances via specified routes (at the specified frequencies and maximum durations) followed by blood sampling at the specified volumes and frequencies then killed. Small molecule PK Studies will generally last for 24 hours although antibody studies may last for up to 2 months.

Implant development in the anaesthetised pig using a non-recovery model

This study type will be performed in the anaesthetised pig for the duration of the study.

Following anaesthetising, the pig will have a number of sham injections performed then the animal killed. This study type will generally last for 4 to 8 hours.

First pass metabolism PK studies using Hepatic Portal Vein cannulated rats

The typical severity experience for animals under this Protocol, will undergo administration of substances via specified routes (at the specified frequencies and maximum durations) followed by blood sampling at the specified volumes and frequencies then killed. Studies will generally last 24 hours.

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

1: ADME/QWBA/PK studies in rodents and lagomorphs (Incl. surgical and non-conventional routes of administration)

The main impact on animals under this Protocol will be confinement within a metabowl for up to 28 days. If the animal undergoes vascular or abdominal surgery there may be some weight loss following the surgery. Analgesia will always be administered prior to surgery minimising any pain post operative.

2: ADME/PK studies in dogs, pigs and mini pigs

The main impact on the animals is the confinement in a metabolism cage for up to 15 days and up to 15 blood samples collected post dose.

No adverse effects are expected under this Protocol.

3: Tissue distribution studies (QWBA) in rodents and lagomorphs (conventional dosing)



The main impact on animals under this Protocol will be confinement within a metabolic cage for up to 28 days.

4: Biliary excretion and enterohepatic recirculation in rat

The main impact on animals under this Protocol will be confinement within a metabolic cage for up to 4 days for the collection of bile samples

5: Milk secretion

No adverse effects are expected on the animals due to the procedures being performed.

6: Placental transfer

The main impact on the animals is the administration by the non-convention route and collection of fetus's under terminal anesthesia.

7: Blood collection by controlled exsanguination

No impact on animals expected as this a terminal procedure.

8: Tissue distribution studies (QWBA) and PK in tumour bearing rodents

Typically an animal will have either an orthotopic mammary tumour or subcutaneous tumour grown for around 14 days. A test material administered then sacrificed via non-schedule one method for QWBA assessment.

9: Conventional PK studies in rodents and lagomorphs No adverse effects are expected.

10: Implant development in the anaesthetised pig using a non-recovery model No adverse effects expected as this a non-recovery Protocol.

11: First pass metabolism PK studies using Hepatic Portal Vein cannulated rats No adverse effects are expected.

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)?

The expected severities under this Project license are mild and moderate.

Protocol 1: The expected severities for guinea pigs, rabbits, rats and mice under this Protocol are 80% mild and 20% moderate

Protocol 2: The expected severities for dogs, pigs and mini pigs under this Protocol are 90% mild and 10% moderate

Protocol 3: The expected severities for guinea pigs, rabbits, rats and mice under this Protocol are 80% mild and 20% moderate

Protocol 4: The expected severities for rats under this Protocol are 80% mild and 20% moderate Protocol 5: The expected severities for rats and neonates under this Protocol is 100% moderate

Protocol 6: The expected severities for rats and rabbits under this Protocol are 90% mild and 10% moderate

Protocol 7: This a terminal non-recovery protocol. The expected severities for guinea pigs, rabbits, rats and mice under this Protocol are sub threshold

Protocol 8: The expected severities for rats and mice under this Protocol is 100% moderate



Protocol 9: The expected severities for rats, mice guinea pigs and rabbits under this Protocol is 100% moderate

Protocol 10: The expected severities for pigs under this Protocol is 100% sub threshold as this a non- recovery model

Protocol 11: The expected severities for rats under this Protocol is 100% moderate

What will happen to animals used in this project?

- Killed
- Kept alive at the establishment for non-regulated purposes or possible reuse.

A retrospective assessment of these predicted harms will be due by 16 March 2030

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, PK and ADME studies are also required in the appropriate species, to support the development of new medicines.

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

In vitro and in silico evaluations are increasingly being used to provide predictive information and where such information is available this leads to replacement of animals. For example, in some situations, the use of ethically procured human tissues (e.g. liver and skin) can provide information directly applicable to humans without recourse to initial evaluation in animals. We offer this service to clients.

We also perform in-vitro fast through-put screening in all species which allows for only promising candidates to undergo animal testing. We always promotes in-vitro testing prior to conducting any studies with animals. This allows for only the promising candidates to undergo animal testing which ultimately reduces the number of animals we use.

Why were they not suitable?

Despite advances in non-animal methods it is still required to use animals where there are



no viable alternatives in order to enable additional much needed advances in medical knowledge and development of new treatments.

We offer a broad range of in-vitro service offerings and where possible will ensure these are deployed instead of conducting animal studies.

A retrospective assessment of replacement will be due by 16 March 2030

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 we are a Contract Research Organisation and offer services to the Pharmaceutical and Biotech industries we can estimate the number of animals we use based on previous years usage.

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

Great care is taken to minimise the number of animals used through study design. We always use the minimum number of animals required to provide robust scientific data which can then be used and accepted by international regulatory guidelines for the development of new treatments.

Rodents and lagomorphs are used once which leads to a higher number of animals being used.

We intend to use up to 500 pigs and 100 mini pigs primarily for PK projects and on rare occasions on a re-use basis. The typical study design would be a 3 Phase study dosed at weekly intervals with blood samples being collected via direct puncture of a jugular vein, following completion of the last sample being collected, animals will be sacrificed. We intend to use up to 130 dogs over the five year period.

Animals that have been kept alive and maintained under the supervision of the NVS may be re-used in this protocol. Each case requires approval from a veterinary surgeon, with knowledge of the lifetime experience of the animals, of fitness of animals to remain alive at the conclusion of the previous study on the basis that: (a) the animals are not suffering and are not likely to suffer adverse effects as a result of the regulated procedures; and (b) that their general state of health and well-being is likely to have been fully restored following the completion of the regulated procedures. Animals that have suffered no more than mildly or moderately during the course of procedures and which are not suffering or likely to suffer as a result may be kept alive in accordance with Standard Condition 11.



Prior to any project initiating we actively seek out relevant information regarding the test item and checks the study design to ensure the least number of animal are used while still achieving the scientific objectives. If group sizes <3 are requested the AWERB will rigorously check the study design to ensure the use of larger group sizes is justified, if this cannot be justified an n of 3 will be used.

Typically the following number of animals will be used on specific studies:

PK and ADME plasma collection studies in rodents and lagomorphs will generally consist of three animals per time point per sex although where possible this will be reduced to two in consultation with the client and provided that the data produced is robust to allow the objective of the study to be met. The reduction to two animals per route/sex is primarily for pharmacokinetic studies and is generally sponsor specific.

PK and ADME studies in dogs will generally consist of three animals per route/sex. Following a PK study the data can be interpreted by performing PK statistics such as power calculations, maximum plasma concentration and exposure. This is generally accepted using as n of three for early stage work by authorities.

Prior to an ADME 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.

The group sizes above are generally 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.

Reduction in the prior license.

Examples of strategies to reduce the numbers of animals and variability that have been implemented by us during the prior license and will be used in the proposed studies are:

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

The use of cassette doses (using multiple compounds in a single dose formulation) reducing the overall numbers of rats used. This is primarily used when assessing PK properties of a compound in rodents. It allows the dosing of multiple compounds to one group of animals significantly reducing the overall number of animals required.

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

The following key references support the need animal numbers in study design related to this project.

OECD Guidelines for the Testing of Chemicals, Test no. 417 Toxicokinetics



ICH Nonclinical Evaluation for Anticancer Pharmaceuticals S9, dated 29 October 2009.

FDA Safety Testing of Drug Metabolites Guidance for Industry, dated November 2016
ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, dated June 2011.

Ballard P. et al. (2012) The right compound in the right assay at the right time: an integrated discovery DMPK strategy. Drug Metab. Rev. 44(3), 224-252

Summerfield S., & Jeffrey P., (2009) Discovery DMPK: changing paradigms in the eighties, nineties and noughties, Expert Opinion Drug Discovery, 4:(2), 207-218

The UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No. 3106) and subsequent amendments. OECD Principles of Good Laboratory Practice (Paris 1998) 11. EC Commission Directive 2004/10/EC of February 2004

10. OECD 427, Skin Absorption In-vivo Method adopted 2004

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

Where possible for rodents and lagomorphs, and as long as the burden to the animal is not too great, each animal will have multiple endpoints to reduce the overall numbers of animal used on a study. For example where animals are placed into metabowls for the collection of urine and faeces, following collection of the final sample the animal would be used for a QWBA carcass assessment as this would not increase the burden on the animal but reduce the overall numbers of animals used on the study, as less animals would have to be used in the QWBA phase. No additional procedures would be performed on the animal to enable it to be used for QWBA assessment.

When the animals are procured generally a certain number of additional animals (~5%) are procured for each study in case of any mis-dosing, such as part of an intravenous dose going subcutaneous or part of an oral dose refluxing into the mouth. Any animals that are not used, or received any dose related material, will be cardiac bled under general anaesthesia to provide control plasma to set up bioanalytical method's within the company. This will reduce the requirement for buying in control plasma which ultimately reduces the total number of experimental animals used in the UK.

A retrospective assessment of reduction will be due by 16 March 2030

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.

Surgical cannulation of blood vessels is performed to facilitate dose administration and in rare cases blood sampling. The rat is used for this model as it is the species used to generate the samples following dosing. Rats are easier to surgically work on compared to mice due to their larger size. The cannula is flushed daily with heparinised saline to ensure patency. Following dosing animals will generally be sampled out to 24 hours post dose following which they will be sacrificed. This procedure normally takes 15 to 30 minutes to perform.

Surgical cannulation of the small or large intestine is permitted. Cannulation of the GI tract for administration of substances, is at present the only method available for reliably delivering the drug to different areas of the GI tract. The use of pH resistant capsules is employed wherever possible although this technique gives variable results depending on the site at which the capsule deposits.

Following dosing animals will generally be sacrificed 24 hours post dose. This procedure normally takes around 30 minutes to perform.

Administration by the intra articular route (rabbits, rats and mice) is predominantly used for compounds which are designed to exert their pharmacological effect within the knee joint. Because the test molecules are generally designed to be restricted to the knee joint they are released gradually, and animals may go out to ~100 days until sacrifice. The animals will be closely monitored for clinical signs, although over the course of us conducting this procedure none have been observed. Drug administrations are performed on anaesthetised animals, using an insulin syringe to minimise the risk of damage to the knee joint by using small needles (27 to 30G). This procedure normally takes around 15 minutes to conduct which includes recovery time. The administration of material into both knees can be required to increase the administered dose, and to allow analysis of different analytical end points using a reduced number of animals.

Intravitreal administration, in the rabbit only, allows the direct administration of substances into one or both eyes. This mimics the procedure employed by physicians in man and currently no other alternative method is available to deliver the compound directly to this site. Analgesics will be administered if deemed necessary and the smallest insulin needle used to minimise the potential harm caused to the animal. Following dose administration the animals will be constantly monitored for at least 1 hour post dose to ensure no signs are observed. The dosing and recovery period normally takes around 20 minutes. Subcutaneous implantation of a slow release device allows the slow release of a compound in the conscious animal. The only alternative is to cannulate the animals and restrain them in a metabolic cage while being infused, using a purpose made jacket and tether. These studies go out to a maximum of 7 days post dose with close attention paid to the site where the mini pump is located to ensure no skin stretching or discomfort to the animal is caused. This procedure normally takes around 30 minutes to conduct.

Instillation via the trachea in the anaesthetised animal, primarily rats, allows direct placement of the compound into the trachea and lungs to assess PK and ADME parameters. This is currently the only method available and provides safety to people working with this technique as it minimises exposure of the compound in the air. Following dosing animals will be sacrificed within a 7 day period. This procedure will always be performed by at least two people with one person being dedicated to the recovery of the



animal after dosing. If any signs of breathing difficulty are observed oxygen will be delivered via a face mask.

Instillation via the trachea in the dog only allows direct placement of the compound into the trachea and lungs. When this procedure is performed in the dog the NVS will be present.

Instillation via the trachea normally takes around 5 minutes to administer followed by a recovery period of about 15 minutes.

Collection of saliva in the dog is a non-invasive method causing very little harm to the animals. Placement of a swab in the mouth for up to 30 minutes could cause some minor irritation to the animal, to mitigate any discomfort and so the dogs do not swallow the swab they will be constantly monitored.

Intra nasal administration is predominantly performed in the rat, mouse or dog. To mitigate any distress very small volumes are administered and the animals are constantly monitored post dose to ensure full recovery. This procedure lasts around 2 minutes with any recovery time.

Topical administration will only be performed on rats and consist of a single application to a pre defined area on the rats back. Rats will be closely monitored for signs of irritation such as a rash. If a rash is observed advice will be immediately sought from the NACWO and NVS. If any rats are kept overnight following dosing, with a plastic o-ring in place on their back, an evening check will be performed although all topical studies performed to date only require the o-ring in place for up to 8 hours. If any rats shows any sign of distress due to the o-ring being in place on the back they will be immediately killed. The dose site will be washed immediately after the rat has been killed to minimise any discomfort caused.

To minimise the chance of interference with the dose site between rats and subsequent ingesting of dose material from the site of application they will be singly housed following dosing until sacrifice. Rats will be given environmental enrichment such as paper wool and aspen balls but they will not be given fun tunnels due to the o-ring rubbing on the fun tunnel and becoming displaced. Rats will be housed on the same rack so they have visual and audio access to others. For these study types rats will generally be singly housed for up to 48 hours but not greater than 7 days.

For rats only, the intravenous dose volume will be increased to a maximum of 12.5mL/kg when the dose is administered as an infusion via a calibrated infusion pump over a 5 to 15 minute period, directly into a lateral tail vein or jugular vein cannula. This increase in dose volume is to ensure the welfare of the animal and to eliminate any signs that could be seen if the dose was to be administered over a shorter period of time. If any adverse effects are seen during the infusion it will be immediately stopped.

For rats only, the intravenous dose volume will be increased to a maximum of 20.0mL/kg when the dose is administered as an infusion via a calibrated infusion pump over, over a 60 minute period directly into a lateral tail vein This increase in dose volume is required to achieve the target dose level due to the compound having a maximum solubility of 1mg/ml. If this was administered at a higher dose concentration over a shorter time period the compound may fall out of solution when entering the blood stream potentially causing respiratory challenges. If any adverse effects are seen during the infusion it will be immediately stopped.



Both of these intravenous dose volumes are higher than the recommended dose volumes (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.). Prior to using these intravenous volumes all previous work performed on the compound will be reviewed to ensure the study design will not cause any harm to the animals. Any reports pertaining to this volume/compound and vehicle, including its pH, will be carefully reviewed and discussed by the AWERB including the NVS before proceeding. We will ensure the smallest dose volume possible is used whilst still achieving the study objectives.

These larger than recommended intravenous dose volumes will only be used where an improvement in animal welfare will be achieved, compared to administering a smaller volume, but as a general rule intravenous dose volumes will be limited to 5mL/kg as per recommended published guidelines.

For rats only, the maximum allowable intravenous infusion time via a jugular vein cannula will be 96 hours. During this period rats will have free access to food and water and be constantly observed during the infusion period. This is only allowed to be dosed to each rat on one occasion. This increase to the infusion time is required to evaluate steady state concentrations and cannot be performed in a more refined method.

The infusions will be administered via a jugular vein cannula with the rats being singly housed in individual metabowls during the infusion period.

For dogs only, the intravenous dose volume will be increased to a maximum of 5.00mL/kg when administered over a 15 minute period infusion directly into a cephalic vein via a calibrated infusion pump through an indwelling cannula. If any adverse effects are seen during the infusion it will be immediately stopped.

In Protocol 2, oral doses maybe administered for up to a maximum of 14 consecutive days. We have extensive experience of dosing by the oral route. All animals will be weighed at least 3 times during the dosing period on days 0, 7 and 14 and increased monitoring deployed through evening checks if required. If any adverse effects are seen during dosing that cannot be controlled, and after consultation with the NVS and NACWO, dosing will be stopped.

Also in Protocol 2, multiple subcutaneous doses (a maximum of three) maybe administered on one occasion per week. These injections will be performed using insulin syringes to minimise any momentary pain the animal might receive. The site of administration will be regularly checked, especially on the day of dosing, and if required an evening check will be performed. This procedure will allow the assessment of three compounds worth of PK data whilst only taking one series of blood collections. Significantly reducing the number of blood sampling required. Where possible cassette dosing will be employed to further minimise needle sticks although this may not be applicable for some compounds due to drug-drug interactions.

Enterohepatic circulation refers to the circulation of biliary acids, bilirubin, drugs or other substances from the liver to the bile, followed by entry into the small intestine, absorption by the enterocyte and transport back to the liver. When performing this study type artificial bile salts will be infused into the duodenum when bile samples are being collected.

The number of blood samples allowed to be collected from rats will be increased to 15 post dose, and 1 pre dose, on very rare occasions when they are required to fully characterise the elimination phase of a molecule with a long plasma half life. This will only be allowed



when an indwelling cannula is inserted into a lateral tail vein to collect a minimum of 8 post dose blood samples. This will limit the number of venepunctures each rat can receive to 7. This is an exception and generally only 12 blood samples will be collected from rats post dose. The total volume of blood collected will not exceed the NC3R guidelines.

The total number of blood samples to be collected from each rat on first pass metabolism studies only is 16 (Protocol 11). First pass metabolism studies require the collection of blood samples simultaneously from both the systemic and hepatic systems, hence the requirement for an increased number of samples. This will be performed by collecting systemic blood from an indwelling catheter implanted into a tail vein and hepatic blood from the hepatic portal vein cannula. This will allow the direct comparison of drug concentrations in each system allowing the first pass metabolism to be calculated. All blood volumes collected, for both the hepatic and systemic systems, will be within the NC3R's guidelines. Needle sticks on this study type will be very low as the hepatic blood sampling is performed via a hepatic portal cannula and the systemic sampling from a tail vein cannula. Both the hepatic and systemic cannula's will be primed with heparinised saline following blood collections to ensure patency of the cannula's is maintained.

Collection of bile in the conscious rat is a method that allows the continuous collection of bile over a time course from animals that do not have a gall bladder. During this procedure the animal has free access to food and water. This method uses less animals than for example taking bile from the gall bladder of a mouse as this design uses a minimum of three animals per time point, not three animals for the entire study. This alternative method also cannot guarantee that bile will be available in the gall bladder after sacrifice which is why a larger group size is required. The rats are on continuous recirculation of bile from the liver into the duodenum right up to the point the pre dose sample is collected on the morning of dosing. While bile is collected prepared bile salts will be infused into the duodenum to replace the bile being sampled from the animal. Animals are placed into purpose made metabowl for the collection of bile, urine and faeces up to 4 days post dose.

Generally bile, urine and faeces are only collected up to a maximum of 72 hours post dose. If a study requires bile collections out to 96 hours specific justification will be obtained from the client to justify this extended collection period. Animals will be assessed for their suitability on study prior to being dosed. Whilst in a metabowl they will have free access to food and water. It will be insured that bile is flowing freely and bile salts are being infused into the duodenal cannula prior to any dosing. The bile salts are formulated in physiological saline giving the animals additional hydration. The cannula attached to the bile duct is exteriorised at the nape of the neck and attached to a purpose made tether and swivel held by a clamp on the top of the cage, to ensure free movement at all times. The bile cannula is checked for the production of bile at least thrice daily but in reality this is done at every sampling time which can be every hour. If the bile is observed to be not flowing for a period longer than one hour the cannula holding the bile will be disconnected and flushed with saline to ensure there is no blockage. If for any reason the bile stops flowing for a prolonged period or the animal appears jaundiced it will be removed from study and sacrificed. Care will be taken to ensure the cannula do not become tangled around the clamp which will be mitigated by taping any excess cannula to the tether so it cannot become tangled. If required wet diet will be given.

When dosing by the intra peritoneal route in pregnant rats or rabbits great care will be taken to ensure no damage is caused to the reproductive organs of the mother or administration to the foetuses. The needle used will have a needle guard to ensure the dose cannot be dispensed too deeply in the body. This procedure will be performed by a



minimum of two PIL holders so the pups can be palpated to the side whilst the dose is being administered. Prior to administration the position of the needle will be confirmed by pulling the needle plunger to access where the needle is. If amniotic fluid or bloods are seen the animal will not be dosed. This procedure will be conducted over a 1 minute period although the dosing only takes around 5 seconds.

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.

Whilst the animals are in metabowls, with expired air being collected, there is an increased risk of the animal asphyxiating due to the pump drawing the air through stopping. To mitigate this happening the pumps are on specific electrical sockets (coloured blue) which are linked to a UPS battery supply. This ensures that if a power cut occurs the pumps will continue running for a minimum period of three hours, which is sufficient time for a PIL holder to get to work and remove the air traps. We also have a diesel generator which starts when a power cut occurs returning normal supply which is tested on an annual basis.

All tumour bearing mice are housed in IVC caging and cleaned out in a change station to minimise the risk of exposure to pathogens that maybe harmful to immune compromised mice. We also use conventional mice where possible for this study type. Environmental enrichment will be given in the form of fun tunnels and paper wool with sawdust on the floor. This enables nude mice to regulate their body temperature more easily. All food and water are irradiated. Syngeneic models will utilise immuno- competent animals.

Sunflower hearts will be given to animals where required to help maintain their weight. All animals will be checked at least twice daily.

Tumour cells maybe inoculated into a single site either on the flank or orthotopically into the 4th inguinal mammary gland.

When the tumours become palpable they will be calliper measured by dedicated personnel on at least twice weekly intervals but normally thrice weekly.

Subcutaneous tumours do not generally metastasise but if this was to be observed the animal would be immediately sacrificed. This will be assessed by the use of clinical signs such as bodyweight changes (bodyweight loss > 15%, compared to weight at inoculation), locomotor challenges, impaired breathing or jaundice.

As we have extensive knowledge of different tumour type growth rates, it can ensure that the animals are typically dosed when the tumours are between 300-700mm³, which will ensure the objective of the study can be met. Any animals exceeding a mean tumour size diameter of 1.2cm will be immediately killed. Previous growth curves will be used to ensure size guidelines are not exceeded.

Genetically altered rodents will be used where this is the pharmacological model of choice or where useful PK information can be gained. In all cases the adverse effects of the altered phenotype are expected to be no more than mild.

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



The choice of species used in this Project are effectively directed by Regulatory Guidance's and/or in early development phase will relate to prior pharmacology/safety/efficacy models and ensure that the species used are the most refined for the intended purpose. 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 such as a rodent prior to using any higher order species such as the dog or pig. If limited rodent data is available we will ensure that the dose levels selected minimise the potential clinical signs which may be seen in a dog or pig.

The international guidance for non-clinical safety assessment mandates two mammalian species including at least one non-rodent species, which includes dogs or pigs be used.

The decision on the selection of the species used in safety assessment studies is made as the initial repeat dose toxicology studies are initiated and should make due consideration of comparative metabolism. The choice of species used on ADME studies will follow that already used. In practice this means rat and mice for the rodent species. The non-rodent species used include dogs and pigs. In some instances the choice of species will be dependent of efficacy models and specifically what species of animals are used in studies of this type. It is critical to understand metabolism in the toxicological species to ensure that the safety assessment data obtained fully covers human exposure of the drug and its metabolites.

We will always aim to use the least sentient animal where possible.

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

All surgeries performed by us are conducted in a class 2 cabinet to increase the sterility and reduce post-operative infections. The surgical equipment is sterilised prior to use via an autoclave and the surgical area cleaned prior to and post-surgery.

All animals are closely monitored post-surgery with animals being placed into a warming chamber at ca.30°C to aid recovery. All animals are weighed daily following surgery to give an additional indicator on health.

Where surgeries are performed an evening check will be performed.

We will always group house rodents and lagomorphs where possible apart from:

- Where the scientific objectives of the study will not be met by doing so, such as single housing in a metabowls
- On welfare grounds, for example if an animal is being bullied
- Following surgery when a companion animal could interfere with the surgery performed
- On QWBA studies where the animal selected for sacrifice at the last time point will be singly housed for up to 7 days
- In all instances of single housing, all animals will be housed on a rack adjacent to other animals of the same species to ensure they have visual and audio access
- Whilst animals are housed in a metabowl, with the collection of expired air, animals will have two checks during that day



If any adverse effects are seen following dosing that persist during the day an evening check will be performed. This will help ensure that any deterioration will be seen and the animal killed to prevent suffering.

We employ a dedicated team of highly trained Personnel License Holders. They are highly proficient at performing the procedures ensuring that the least distress is caused to the animal when conducting any procedure.

If we undertake a new procedure it will be ensured that the Named Veterinary Surgeon is present on the first occasion of performing the new procedure.

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

We are a GLP accredited facility and as such perform all studies to GLP if regulated or to the spirit of GLP if non-regulated. This ensures the procedures are recorded and performed according to company Standard Operating Procedures.

We will always collect samples and administer treatments according to guidelines provided by the NC3R's and LASA, unless specifically authorised within this license.

We ensure that all our caging and environmental conditions conform or are higher than the Code Of Practice.

The following key documents give guidance on the study types we perform.

OECD Guidelines for the Testing of Chemicals, Test no. 417 Toxicokinetics

ICH Nonclinical Evaluation for Anticancer Pharmaceuticals S9, dated 29 October 2009.

FDA Safety Testing of Drug Metabolites Guidance for Industry, dated November 2016

ICH S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, dated June 2011.

Ballard P. et al. (2012) The right compound in the right assay at the right time: an integrated discovery DMPK strategy. Drug Metab. Rev. 44(3), 224-252

Summerfield S., & Jeffrey P., (2009) Discovery DMPK: changing paradigms in the eighties, nineties and noughties, Expert Opinion Drug Discovery, 4:(2), 207-218

The UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No. 3106) and subsequent amendments. OECD Principles of Good Laboratory Practice (Paris 1998) 11.

EC Commission Directive 2004/10/EC of February 2004

10. OECD 427, Skin Absorption In-vivo Method adopted 2004

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

All Personnel license holders go on relevant training/refresher courses and keep up to date with refinements by attending National Centre for the Replacement, Refinement and Reduction of Animals in Research or Royal Society for the Protection of Cruelty to Animals workshops or equivalent annually.

We always send personnel licensees to Congress run by the Institute of Animal Technology and the Laboratory Animal Science Association conference. Advances in the 3R's are then discussed at the following Animal Welfare and Ethical Review Body and if everyone agrees, is implemented. An example of this is the use of red rodent houses which are provided to all animals, when not on a study.



For large animals we constantly drive to improve enrichment with treats, toys or new bedding for an example. These are all discussed at each Animal Welfare and Ethical Review Body meeting.

We have active members on the Home Office Liaison Contact Forum group who look for improvements in our welfare practices.

We have sites located globally and periodically have meetings with other animal sites to discuss procedures and any refinements related to them. An example of this is our UK site discussing a more refined method of blood collection in the pig without the need for jugular vein cannulation.

A retrospective assessment of refinement will be due by 16 March 2030

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. The Metabolism and Kinetics of Pharmaceuticals and Medicinal Products

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

Pharmacokinetics, Metabolism, Pharmaceuticals, Rodents, ADME

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant
Rats	neonate, juvenile, adult, pregnant
Rabbits	neonate, juvenile, adult, pregnant
Beagles	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.

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 aim of this project is to perform metabolism and pharmacokinetic studies to support the development of Human and Veterinary pharmaceuticals. These tests, commonly referred to as ADME studies, are designed to investigate the **A**bsorption of test substance related material (how substances get into the blood), **D**istribution around the body,



(including into blood and tissues), pathways of **Metabolism** (how the body breaks substances down), and routes and rates of **Excretion** (how the body removes the substance), by harvesting samples (blood, plasma and other bodily fluids, tissues and excreta) at different times after administration.

The data will be used to review substances under development or satisfy governmental requirements necessary for approval of clinical trials (dosing in humans or animals).

A retrospective assessment of these aims will be due by 19 March 2030

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 primary benefit of work carried out under this licence will be to allow regulatory authorities (who are totally independent from the commercial interests behind every marketing application) to come to decisions regarding the marketing of new compounds based upon safety data generated in these studies relating to exposure (pharmacokinetics) and ADME (metabolism) characteristics of the test compound.

This will provide data supporting the rate and extent of absorption; distribution into tissues; key enzymes involved in metabolism; and the rates and routes of elimination; to assist in scaling safety margins of exposure and toxicity into man.

The introduction of new pharmaceuticals and veterinary pharmaceuticals is beneficial because of associated improvements in the health and welfare of humans and animals.

There is also a major benefit when including screening experiments, as the most promising compounds for further development will be selected and then fully tested so that they can reach the market sooner. This will ensure subsequent benefits in both the health and welfare of humans.

The use of the experiments described in this licence may indicate major safety limitations with the development of the substance under evaluation at an early stage thus precluding requirement for additional experiments after these screening studies.

This can greatly reduce the number of animals required in a programme of work. In addition, scientific knowledge gained in one programme of work will often be applied to future experiments in order to reduce animal numbers and/or reduce pain and stress to those animals used in the subsequent work, or to target investigations to a particular organ or tissue during toxicity testing or clinical trials.

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



The principal benefit of the project is the provision of pharmacokinetic/toxicokinetic pharmacodynamic and ADME data to facilitate sound development or regulatory decisions on such as refinement of the development strategy, clinical trial approval or marketing authorisation for new medicines.

For pharmaceutical compounds in human and animal medicine we will provide information to European and worldwide regulatory bodies which will allow sound development or regulatory decisions on the development strategy, clinical trial approval or marketing authorisation for new medicines. We will provide data on pharmacokinetics, absorption, distribution, metabolism and excretion (ADME) of such compounds to allow both customers and regulators make decisive 'go' or 'no go' decisions regarding future testing (in animals and man). These studies will be performed to satisfy International regulations.

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

Patients will benefit from these studies as this work will contribute to the development of new drugs. These new drugs may work better in the clinic, relieve or cure diseases and have better side effect profiles. We may, by our work, also contribute to better knowledge and understanding of these types of drugs, and that knowledge may be used to develop further new drugs.

Our customers will also benefit, as the data we generate will allow them to progress their new drugs into clinical trial, make decisions on their development programmes, or prevent further development if they are found to have adverse pharmacokinetic or metabolic profiles.

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

The work will be shared with customers who will use it to determine their future strategy, or for submission in documents required by regulatory authorities. Whilst we have no direct control over what happens to the data after we have shared it, we trust from information given to us that it is used to support candidate selection or to support drugs progressing to clinical trials. Where appropriate, we collaborate with our customers to share data we have produced in the form of scientific publications that are in the public domain.

We are able to advise our customers on which studies are required in their development programme and on suitable study designs, based on our experience and on knowledge gained from previous feedback from customers and/or regulators, leading to focused and effective studies.

It is difficult to predict how the benefits of any work done on this project will be seen in the future due to confidentiality issues. However, this work will contribute to the pharmacokinetic/metabolic profiling and safety of pharmaceuticals that can be administered to humans and animals, either by informing on safety and allowing to progress to clinical trials or preventing pharmaceuticals reaching the market due to pharmacokinetic/metabolic/safety issues), which in itself reduces the overall number of animals used, by preventing further testing.

Species and numbers of animals expected to be used.

- Mice: 6700
- Rats: 16350



- Rabbits: 890
- Beagles: 1290

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.

Rats, mice, rabbits and dogs may be used on this project. The majority of work will be carried out in adult animals, however it may be a requirement to use juvenile or neonate animals to support pharmacokinetic/ADME work for drugs intended for non-adult populations.

Most studies on this project will be done in rats and mice as rodents are the lowest sentient species that have the physiology specifically similar to humans that meaningful predictions of human ADME, pharmacokinetics and pharmacodynamics can be made.

Scientific opinion, including that of the regulatory agencies (who are independent of governments), indicates the use of one rodent and one non rodent species for many of the metabolism and pharmacokinetic studies that are required, to allow drugs to be properly assessed for safety and for potential testing in humans.

The rabbit maybe used when either previous work on a compound has started in the rabbit, or when the use of a rat or mouse is unsuitable due to harmacological/physiological reasons, for example.

The most appropriate non-rodent species (dog or rabbits on this licence) will be selected with reference to studies performed in rodents, evaluating cross-species metabolism and toxicity studies in test tubes (similarities in effects between rodents and dogs), and pilot pharmacokinetic and safety assessment studies. The species selected based upon this information is the one which is predicted to be most similar with human in terms of sensitivity, pharmacokinetics and metabolism. The minipig is considered the primary non-rodent species (these studies are conducted under a different project licence) unless there is specific scientific justification for the use of dogs or rabbits. When considering veterinary products, the drug may be specifically aimed at dogs, in which case it is obligatory to study the dog as the target species. Dogs are only used where the purpose of the programme of work can only be achieved by their use. Juvenile dogs would only be used for specific studies with suitable justification. No neonate dogs will be used.

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

Most of the animal procedures undertaken under this licence would be at a mild severity.

For a standard design, animals will be administered with a test substance (typically oral gavage or intravenous administration) followed by routine collection of blood and/or excreta. Following completion of the sample collection, animals may either be killed by an appropriate method (to allow for tissues or carcass collection) or the animal maybe returned to stock for re-use following a suitable veterinary examination (typically dogs).

Specific procedures may require confinement or restraint, but this is minimised wherever



possible. Excreta collection generally required single housing to meet the objectives of the study, but this again is kept to the minimum period required to meet the study objectives (typically 1 week). Where possible, animals will be habituated to unfamiliar equipment or procedures to minimise stress.

Routine collections of blood will generally be taken by venepuncture of a peripheral/superficial vessel though implanted cannulae may also be utilised where appropriate.

Studies vary in duration from a single dose to daily dosing for up to 13 weeks, depending on the intended use or likely exposure to each substance under study.

Animals may be subjected to a series of linked regulatory procedures when studying a single test compound, such as cross-over design experiments. In this study type, or series of studies, animals may receive a dose of the test material by one route and after a suitable wash out period, a second dose, either at a different concentration or via a different route. Additionally, where multiple compounds need to be tested, each with a similar mode of action or therapeutic theme, these may be administered in a series of experiments. Most dosing periods, will however, be short term ones (< 4 weeks).

Surgical models (typically biliary and vascular cannulation) are used to allow investigation into biliary excretion, to infuse a dose over an extended period of time, to replace biofluids or to collect serial samples (or any combination of these). For each surgical procedure, appropriate analgesia and balanced anaesthesia will be provided as standard and thereafter as required in accordance with a regime agreed in advance with the NVS.

Animals will be under the care of highly trained technicians who will apply a rigid framework of welfare assessment to allow early detection of animals showing signs of discomfort or distress.

Some, less routinely used study designs, require the collection of tissues under anaesthesia. In these designs, the animal will be killed, under anaesthesia and not be allowed to recover consciousness.

Most rodents will be humanely killed at the end of procedures. However where the study type allows it, (such as blood sampling for pharmacokinetics or metabolic assessment) and subject to a suitable veterinary examination, animals (typically dogs) may be re-used in future studies.

If an animal is suitable for rehoming as a companion animal, after being considered suitable after a veterinary examination, this may also be allowed.

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

When dosing an animal by injection or taking blood, the amount of pain an animal feels is similar to what a patient would feel having an injection done, or having a blood sample taken by a doctor. If we have to repeatedly inject animals using a needle and syringe, we would choose different sites to do this where possible. If we can take blood samples when an animal is unconscious then we do. If we need to take repeated blood samples or need to dose repeatedly then we try and use different sites. Of course everyone who performs these procedures is trained to a high standard.



Animals undergoing surgery receive the same sort of care as a patient would in hospital. We discuss their pain relief and use of antibiotics with a vet before we start.

We administer drugs as necessary and give them plenty of time to recover from surgery before we use them in experiments.

We often need to take a urine/fecal sample for analysis, so we would then put an animal into a special metabolism cage which requires the animal to be single housed. Virtually every animal will get used to their new cage within about 15 minutes and show no signs of distress. Dosing with pharmaceuticals may cause adverse effects in some studies, although this is rare, as the animals are dosed at therapeutic doses (a similar level to what a patient would be dosed at), not at toxic ones. We do observe our animals at least twice a day, and the people who do this know the signs when an animal shows adverse effects.

If an animal does show adverse effects we would check it more frequently, and get more senior staff involved in its care for advice, including vets. We also help these animals out by giving more bedding, more heat and special food to make them more comfortable.

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)?

Under the last project licence, approximately 23% of procedures were performed as non-recovery ones, approximately 69% of animals experienced mild severity, and 8% of animals experienced moderate severity (animals mainly used after surgical preparation).

We would expect similar severities in this licence, however this would be dependent on the nature of the work performed.

What will happen to animals used in this project?

- Killed
- Kept alive at the establishment for non-regulated purposes or possible reuse
- Rehomed

A retrospective assessment of these predicted harms will be due by 19 March 2030

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?

Metabolism and pharmacokinetics are very complicated processes, and although there are



metabolic tests that can be performed in-vitro (without the use of animals), there are no adequate models to replace the whole animal experimental model, as the complexity of the human body cannot be fully replicated in a test tube.

In the case of new pharmaceuticals, the regulatory authorities are obliged to protect human volunteers for clinical trials by requiring use of proven test systems and accepting use of animals until an in-vitro alternative has been demonstrated to be reliable and reproducible.

This includes the study of absorption, distribution, metabolism and excretion of these substances. Information on distribution and elimination is necessary in all cases where such data are indispensable to determine the dosage for humans.

Investigations are continually evolving into pharmacokinetic modelling from in-vitro data, thereby allowing better prediction of human responses and reducing the number of animals needed to generate the required data, but currently these remain limited in their reliability to go beyond indicating trends, due to the nature of complex metabolic processes in-vivo.

In many cases the protocols listed in this Project will be used later in the life cycle of a test substance and in many cases, in-vitro tests will have been conducted previously (often by the Sponsors) to ensure the drugs do what they are meant to, before we expose them to testing in animals.

Where non animal alternatives can be used and are accepted by regulatory authorities, they will be.

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

There are some studies that can be carried out in-vitro that can be used to support ADME work including tests that assess metabolism and absorption of substances, and how well they bind to key proteins in the blood. Predictive software can also be used.

Why were they not suitable?

None of these tests can yet model the complex and integrated mechanisms governing the ADME of pharmaceuticals fully and hence, animal testing is still a requirement. Work is on-going to refine the prediction of human pharmacokinetics from in-vitro data, although this is not currently at a stage where it can replace the animal models.

A retrospective assessment of replacement will be due by 19 March 2030

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 used on this project is estimated from those used under previous projects and after consideration of regulatory trends, and in review of future services being developed and offered for the screening and selection of new molecules for regulatory development studies.

Studies will be designed under this licence such that the minimum number of animals will be used in order to obtain the maximum information, whilst the scientific objectives of each study are met, in accordance with regulatory requirements and agreed standard practices.

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

We have a wealth of experience and knowledge regarding regulatory requirements and the industry standards used, hence we are in an ideal situation to influence study designs when discussing study requirements, and will always consider ways of reducing animal numbers. We also have available professionally trained statisticians to help design studies, if required.

In order to fulfil the requirements of the regulatory agencies, animal group sizes for metabolism studies are variable, depending on the test substance being used, the specific type of study being undertaken and the strain of animals being used. For instance, for most studies involving pharmaceuticals, group sizes will not generally exceed 3, unless a sound scientific rationale is provided.

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

All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals. For study types that are less well established and for which historical data may not be available, the literature (scientific publications) will normally be consulted to help decide the group size. We can also consult with our statisticians, particularly where the study type is not routine and/or complex, as they can use calculations to estimate the correct number of animals needed to meet the objective of the study.

For studies where a new substance 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 levels we chose are safe, and the substance affects the system it is designed to, without making an animal ill. These are called pilot studies.

We will try and get as many outputs as we can from a single animal where possible, without adversely affecting its welfare or compromise the main objective of the study.

Where possible, common control groups are used in order to minimise the numbers of animals used, or control animals are not used at all, where the doing so would not benefit the study design.

The re-use of animals, (see above) under carefully controlled conditions, under the



supervision of a veterinary surgeon, also means the overall number of animals we use is reduced.

A retrospective assessment of reduction will be due by 19 March 2030

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.

Regulatory evaluation of pharmaceuticals generally requires investigation in two relevant mammalian species; usually one rodent and one non-rodent. This project predominately uses rats and mice as rodent test species.

For the non-rodent species, rabbits and dogs are used in this project. Selection of which is most suitable is considered according to the physical, physiological and behavioural requirements of the study, biochemical or metabolic similarities with target species (such as man), similarities of action and response to the compound of study, temperament and robustness in response to blood sampling. Dogs are only used where the purpose of the programme of work can only be achieved by their use. The minipig (conducted under a separate project licence) or rabbit would be used otherwise.

The rabbit shares many characteristics with rodents but are considered a non-rodent choice when it comes to selection of a second test species to meet regulatory requirements. The minipig is considered the primary non-rodent species (these studies are conducted under a different project licence) unless there is specific scientific justification for the use of dogs or rabbits.

All animals are monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (such as modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).

Highly trained staff use a rigid framework of welfare assessment to allow early detection of animals showing signs of discomfort or distress. We use pain relief as standard with anaesthesia and after procedures where relevant such as surgically implanted models.

We sometimes, although rarely use animals that have had their genetic material altered such as that they are predisposed to developing a condition or if a particular gene is important say in a specific component of the ADME process in which we have interest.



Good Surgical Practice, including aseptic technique (Laboratory Animal Science Association 2017 Guidelines) will be observed for any animal undergoing surgical procedures, as well as the provision of pain relief and antibiotics, when needed.

Environmental enrichments appropriate to the species are used within the animal facilities, such as play areas/toys, chew-items etc and except where the scientific objectives of a procedure or animal welfare considerations prevent it, all animals are group housed, so that they can interact and behave socially wherever possible.

Study designs are reviewed, and new methods considered as technology best practice and standards improve and advances become adopted and approved by international regulatory agencies.

Wherever possible, experimental samples are collected under anaesthesia or post mortem to minimise any potential suffering. In some circumstances safety markers will also be collected from the animals maximizing the data from individual studies. Maximising data decreases use of further animals and collecting samples post mortem or from terminally anaesthetised animals, minimises suffering.

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

Rats and mice will be used in most of the studies conducted under this licence.

Rodents are considered to be the species with a similar enough brain/nervous system and physiology that will allow us to achieve the study aims and are considered suitable for the predicting what's likely to happen in humans.

For other species, there would need to be specific scientific justification provided for them to be used. This may be, for example, that other studies investigating a particular test substance have already been carried out in that species, or there is a particular metabolic pathway that is only available in that species or say that is the target species for the actual test substance.

The minipig is considered the primary non-rodent species (these studies are conducted under a different project licence). However, the use of dogs or rabbits may be more appropriate (for example were the liver enzymes and transporter mechanisms for xenobiotics are yet to be established in minipig) will be driven by the need to fulfil regulatory requirements and to provide appropriate data to support clinical trials in humans.

Some of the studies carried out under this licence will be some way into a development project where the scientific justification for the use of dog may have already been established. In that case the justification for the use of the dog under this licence will still be assessed using the information available.

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

Many of the procedures used in this licence are standardised, well defined and already well refined over many years. We will continue to assess any future possible refinements over the duration of this licence.



Where animals do show adverse clinical signs after dosing or surgery, we will increase the frequency and length of observations, and provide supplementary interventions (like extra bedding/food/heat) where needed, until the signs resolve.

Similarly, if after discussing with a vet and senior technician, we decide an animal is not recovering from procedures, and there is no prospect of them doing so in the near future, we will humanely kill them to prevent further suffering.

Habituation of animals to equipment like dosing tube and metabolism cages will be carried out prior to the start of the dosing phase.

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

Regulatory guidelines

ICH S3B Pharmacokinetics: Guidance for repeat dose tissue distribution studies, and ICH S3A Toxicokinetics: The assessment of systemic exposure in toxicity studies, (June 1995).

DIRECTIVE 2001/82/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 6 November

2001 on the community code relating to veterinary medicinal products.

DIRECTIVE 2001/83/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 6 November

2001 on the community code relating to medicinal products for human use.

ICH Topic M3(R2): Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing authorisation for Pharmaceuticals, ICH (2009).

The OCSPS harmonized test guidelines (OPPTS): Health Effects Test Guidelines; OPPTS 870.7485 (Metabolism and Pharmacokinetics) and 870.7600 (Dermal penetration) (August 1998).

ICH guideline S6 (R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals. FDA Guidance for Industry on the Safety Testing of Drug Metabolites (MIST), March 2020.

Surgical Procedures

LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017)

Blood sampling and dosing

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).

Diehl et al (2001). A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23.

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



We have a dedicated regulatory affairs team that monitor regulatory changes and feed those back to the scientists who do this work.

A retrospective assessment of refinement will be due by 19 March 2030

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. Regulation of bone development, function and structure in health 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

bone, skeleton, mineralisation, inflammation, metabolism

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

My ongoing research programme aims to understand the physiological mechanisms required for bone formation, and how disease states impact on the structure and function of the skeleton.

A retrospective assessment of these aims will be due by 25 March 2030

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 bony skeleton not only provides physical support and protection for vital organs but is also a crucial storage site for bone marrow and essential ions such as calcium and phosphorus. In addition, bone is a highly dynamic endocrine organ producing hormones and growth factors that influence other body systems. Bone mass and quality is precisely regulated by local and endocrine factors and when this regulation is disrupted, a diverse range of bone formation and mineralisation abnormalities result, including diseases of great public health concern, e.g., osteoarthritis, osteomalacia (rickets), paget's disease and osteoporosis. Furthermore conditions that effect other organs in the body such as inflammatory bowel disease (colitis and crohn's disease), Duchennes muscular dystrophy and chronic kidney disease can result in poor bone health through the production of inflammatory cytokines and/or abnormalities on calcium and phosphorous retention. The poor bone health associated with specific diseases can be exacerbated by the drugs e.g. glucocorticoids used to minimise the accompanying inflammation.

In all these cases, the skeletal defects are a result of altered physiological control of bone cells and the proposed studies will provide invaluable basic/translational knowledge that will benefit our ability to offer therapeutic interventions to alleviate the deterioration of bone health.

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

The proposed studies will provide invaluable fundamental/translational outputs in the form of peer reviewed publications, book chapters, seminars, and oral and poster presentations. Outputs may also include data that validates a drugs effectiveness in the treatment of skeletal disorders.

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

The immediate beneficiaries will be members of my own research group and colleagues within the wider University. Through dissemination of data via publications, seminars and conference attendance, other like minded scientists will benefit allowing them to build on observations to extend our knowledge of basic cellular processes or pathological mechanisms.

I work close closely with national and international colleagues where group efforts are made to understand biological mechanisms underpinning bone disease. The data



produced in this project will contribute to the success of these collaborations and in turn lead to further funding opportunities.

Some of my studies are of a fundamental nature where we aim to accrue knowledge of how bone forms whereas others are of a more translational nature. An example of the latter is a study to determine the effectiveness of a drug (gastrin receptor antagonist) to protect bones from the adverse effects of anti-acid treatments used for alleviating the symptoms of indigestion/heart burn. This work on bone loss due to the use of anti-acid treatments has also identified that the inhibition of an enzyme required for bone mineralisation by anti-acid treatments may also be a factor in the poor bone health noted in people taking these treatments. Another translational study will test a novel compound to build bone and alleviate the symptoms of post-menopausal osteoporosis. Both these studies will use rats as the preferred animal model. More fundamental studies using normal and genetically altered mice have identified mechanisms that may be involved in the pathogenesis of some common human diseases. Examples of this include chronic kidney disease (CKD), which affects millions of people world wide and one side effect of impaired kidney function is poor bone health (renal osteodystrophy). Our studies using a mouse model of CKD have identified a novel mechanism in bone cells that may contribute to this pathological condition. Other studies using a mouse model of inflammatory bowel disease (IBD) have identified several mechanisms that may help prevent the adverse effects of this disease on bone health and bone fractures. Glucocorticoids are used as anti-inflammatory drugs in many conditions and are one of the most widely used medicines in the world. Unfortunately, one of the major side effects of their use is bone loss and fragility fractures and this is particularly evident in the elderly and individuals with muscle wasting conditions such as Duchene's muscular dystrophy (DMD). There is a number of potential compounds that can help prevent skeletal disorders due to glucocorticoids and we will investigate these in animal models of DMD. In the longer-term this basic/translational research which will be extended and refined by others may lead to drug targets and treatments of many skeletal diseases. Ultimately this could benefit the health of both humans and animals.

We will also use some of the data to help with our roles in public engagement. This will be facilitated via a programme of hands-on, curriculum-linked workshops for school pupils in P6 to S6, activities for teachers and interactive lab-based sessions for community groups of all ages.

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

This will be mainly through peer-reviewed publications but as I also get seminar invites I will also use these forums to publicise the outputs from this project. Social media and websites will also be used to bring attention to published material. However, maximising the outputs will be a team effort and the post-docs and graduate students within my research group will maximise outputs by attending many conferences to deliver talks and posters to a wide range of scientists in various disciplines.



Species and numbers of animals expected to be used

- Mice: 3500
- Rats: 250

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 skeletal system is a complex organ made up of a variety of cell types and connective tissues. Its function is also controlled by many endocrine factors synthesised and secreted from distant soft tissues. Therefore, a physiological context is required to derive meaningful and robust data when studying bone and cartilage. For this we will use well established animal models such as rodents (rats and mice). Rats and mice also have a skeletal system that closely resembles that of humans in both structure, development and resident cell type. Using genetically altered mice can also be informative about the function of specific genes in normal skeletal development of disease. Mice and rats are therefore the ideal model and we will use all life stages from embryos to adults.

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

The protocols described in this application are designed to achieve the objectives of this licence. The experimental design will use techniques described within the protocols in isolation or in optimal combinations so that we maximise informative data. However the use of multiple procedures on the same animal will be carefully regulated and in the majority of cases limited to the study of one superimposed disease state i.e. ovariectomy (to mimic post-menopausal osteoporosis), chronic kidney disease (CKD), osteoarthritis, inflammation, or glucose manipulation/dietary manipulation with or without the administration of substances to prevent disease or modify skeletal metabolism. Some techniques will be used in more than 1 protocol. The duration of the experiments will vary from study to study but typically last for 4-5 weeks e.g. induction of CKD or ~3 months e.g. effect of anti-acid treatments on the skeleton. In all cases, tissue will be collected after death (unless otherwise stated; e.g. blood sampling from the tail) and subjected to a range of analyses.

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

Animal suffering will be limited in our studies by our strict monitoring of severity limits and our use of protocols that do not produce excessive trauma or suffering. Drugs will be administered at non-toxic



dosages and if unknown, this will be tested in a carefully graded dose-finding protocol. Delivery of substances should involve only slight and transient pain and good injection technique by experienced staff will minimise pain at injection. Similarly, ear notching should involve only slight and transient pain and no healing problems. To avoid the stress of handling animals where long-term repeated administration of compounds is necessary, we will make use of sustained release subcutaneous implants and osmotic mini-pumps where possible

Some of the alternative strategies which involve surgery will be kept to a minimum. Examples of surgeries include ovariectomy (removal of ovaries) to mimic post-menopausal osteoporosis and destabilisation of the medial meniscus (DMM) in the knee joint to mimic osteoarthritis. From our past experience, ovariectomy does not cause any chronic health and welfare issues and the DMM model, which we have used previously, can cause lameness within a 4-day period of surgery but generally not beyond this time period. Analgesics will be administered prior to the surgery to cover the immediate post-operative period. However, any animal showing adverse effects that cannot be relieved by appropriate treatment will be killed by a Schedule 1 method. During observations of animals, particular attention will be paid to respiratory rate, colour of mucous membranes, levels of normal cage movement and grooming and feeding behaviour.

Furthermore, in the CKD model, potential adverse effects will be related to the impaired renal function and include reduced food intake and weight loss. Animals will be reviewed twice daily and body weight will be analysed as will the presence of clinical symptoms using a health score sheet to determine humane endpoints. Additional potential adverse effects may include loss of coat/skin condition, diarrhoea, reduced food/ water intake, abnormal behaviour, hunched posture and poor body condition.

In most cases the adverse effects of alterations of gene function will be minimal.

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)?

Of the total of 3500 mice projected to be used over the course of 5 years, it is expected that the severity experienced by 34% (1190) will be sub-threshold, by 50% (1750) mild, and by 12.5% (438) moderate, while the severity of 3.5% (122) is expected to be severe.

Of the total of 250 rats projected to be used over the course of 5 years, it is expected that the severity experienced by 50% (125) mild, and by 50% (125) moderate.

What will happen to animals used in this project?

Killed



A retrospective assessment of these predicted harms will be due by 25 March 2030

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 aims of this PPL require a physiological context and therefore an in vivo approach will be used and mice will be the experimental model in most cases, although rats will be used in some protocols .

Indeed the use of live animals is essential to model human diseases and to make it possible to monitor bone development, formation and structure by imaging e.g. x-rays and histological procedures. Many of these studies also require blood and urine biochemistry analysis and these measurements are not possible in an in vitro model.

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

In vitro cell culture (cell lines and primary cells) and bone organ culture approaches will be used where it can -

- Provide preliminary data to test out an idea/hypothesis before considering more in depth animal models.
- Offer advantages e.g. to remove confounding variables of changes in systemic parameters.

Why were they not suitable?

It is recognised that in vitro approaches have a number of limitations and do not mirror what happens

in vivo. Examples include -

- In vitro cell cultures do not represent all in vivo tissues and as such translation of data from cell culture experiments to human and animal physiology/health is problematic
- There is a loss of inter-cell and inter-organ interactions; thus indirect effects of agents on bone physiology are not readily detectable in vitro.
- Many tissues in vivo are quiescent (non-dividing), whereas cell lines are continuously proliferating.
- There is questionable correspondence between the in vitro response to the in vivo response. This is often due to loss of the differentiated phenotype in culture.



Also, the study of genetically modified mice is essential for some studies and no alternative animal species is readily available. Knocking out or overexpressing the activity of a gene provides information about what that gene normally does in a physiological context. This information cannot be obtained from in vitro models where, for example, cell-cell interactions and systemic regulatory pathways are lost. However once information has been obtained from these in vivo models, all efforts will be made to minimise the number of mice used by carrying out well designed in vitro experiments using cell lines or cells from the wild-type and genetically altered mice.

A retrospective assessment of replacement will be due by 25 March 2030

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 to be used are based on:

- Past experience which I have accrued over many years as principal investigator and PPL holder (since 2004).
- The studies to be completed on currently funded grants.
- The likelihood that further funding will be secured.

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

All protocols have been designed so that where possible, a number of different analyses can be completed from the same animal sample, thus minimising animal usage/maximising output in keeping with the NC3R's. Previous experience in study design will be utilised but as is the case now, I will also consult the NC3R's Experimental Design Assistant (EDA) to ensure that we use the minimum number of mice/rats consistent for each scientific objective. The EDA will also be used to help reduce experimental bias i.e. blinding and random allocation of treatments and provide guidance on appropriate statistical analysis of the resulting data. I have also attended the annual Institute seminars (and viewed also on line) on good statistical analysis and experimental design given by the Institutes Statistical Advisor.



All sample numbers/biological replicates for each set of experiments to be performed on rodents will be subject to a sample size calculation informed by our published data sets on changes in bone structural parameters. In most cases we will use an alpha (significance) of 0.05 (5%) and power of 0.90 (90%) to determine animal numbers in each experimental group to ensure we detect statistically meaningful differences (10% changes in bone parameters). Advice on the proposed experimental designs and methods of analysis of the results will be taken from our Institutes Statistical Advisor

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 to be used will be optimised by:

- When breeding animals for a specific study the minimum number of mice will be generated from mating pairs.
- Where a new procedure or animal model is to be used, a pilot study with a small number of animals will be designed to ensure that the procedure and/or interventions are appropriate for the research question asked. We will review and where appropriate refine new procedures based on the data obtained to ensure the benefits outweigh the harms. This strategy will also highlight adverse effects of novel compounds administered.
- Where scientifically appropriate the sharing of tissue among members of my group will be encouraged to minimise animal use.
- If appropriate pilot cell culture studies will be completed first to provide proof-of-concept before embarking on an animal study.

A retrospective assessment of reduction will be due by 25 March 2030

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.

Our decision to focus mainly on mice as the rodent of choice has been made to provide us with the potential to explore the role of specific genes in the responses we identify, through the use of mutant and gene edited mouse models. However, in specific studies rats will be



used as they are a better model to study post-menopausal osteoporosis and the effects of proton pump inhibitors, hypoacidity and hypergastrinemia on bone health.

Specific models and methods

- The invasive procedures described in protocols 3, 4 and 5 (all moderate) e.g. to determine insulin function, to induce chronic kidney disease (CKD), inflammation or inflammatory bowel disease (IBD) and in vivo joint loading have been developed for mice and have been widely used in this species by scientists both in the UK and overseas. Precise schedules to complete these procedures have been obtained and refined during the current and previous PPLs. For example, the amount of insulin administered for an insulin tolerance test will use previously established dose rates that does not result in hypoglycemia.
- Pilot studies have confirmed that the optimum concentration and duration of dextran sodium sulphate (DSS) to induce IBD is 3% DSS in the drinking water for 4 days. Only a few mice on this regime lost more than 20% of their body weight and required to be culled in accordance with the Home Office animal licence regulations. We have considerable evidence that mice treated with this regime recover well after removal of DSS.
- Mechanical loading procedures have been fine-tuned by our colleagues and collaborators and have been used successfully on mice within our small animal unit. Therefore no pilot expts were required. Loads applied will induce strains in the higher range of those experienced physiologically. Soft tissue damage during application of the loading is avoided by using polished metal or padded supports as appropriate. Analgesia is not considered necessary after loading.
- Surgical inducement of osteoarthritis is a procedure we have successfully used in previous PPLs and no unexpected events were noted. Lameness within 4 days of surgery is not unexpected but we do not expect to see any lameness after this time post surgery. Any animal showing adverse effects that cannot be relieved by appropriate treatment will be killed by a Schedule 1 method. During observations of animals, particular attention will be paid to respiratory rate, colour of mucous membranes, levels of normal cage movement and grooming and feeding behaviour. Analgesics will be administered prior to the surgery to cover the immediate post-operative period.
- Our previous unpublished studies were able to identify a dose of lipopolysaccharide (LPS) to induce inflammation that resulted in altered linear bone growth but without any negative impact on the behaviour or other welfare indicators. However, others have noted transient signs of lethargy, piloerection, and a hunched posture. Therefore, all animals will be observed daily for any unacceptable signs of discomfort or distress
- In muscular dystrophy studies the use of the grip strength test in mice will follow the standard operating procedure devised by the TREAT-NMD collaboration (www.treat-nmd.eu/downloads/file/sops/dmd/MDX/DMD_M.2.2.001.pdf) to minimise any risk of harm or adverse effects to mice. The forelimb bones from the first animals tested in this way will be examined as part of their skeletal phenotyping, which will allow us to confirm whether the test has had any adverse effect. Grip strength has been determined in many cohorts of mice during the previous licence and no adverse effects have been recorded. However, any animal suffering will be minimised by



regular monitoring by trained staff, using monitoring systems specifically developed for each model/procedure, and application of well-defined humane endpoints.

- The adenine supplemented diet strategy to induce CKD in mice has been recently refined as reported in various publications. This will be used in protocol 6 (severe). High adenine doses (0.75% and 0.3% are lethal) whereas 0.125% did not result in elevated urea levels. In contrast diets containing 0.2% adenine did promote high urea levels and CKD but limited mortality in grouped housed mice.

Our published studies to date have informed us that a 0.2% adenine diet fed for up to 5 weeks is achievable and induces many of the serum and urine factors characteristic of CKD. Bone changes are also observed confirming that CKD results in the impairment of bone health. Longer durations on a 0.2% adenine diet resulted in excessive weight loss (30%) and the mice had to be culled. We have however noted excessive and rapid body weight loss in mice that have been singly housed (for pair-feeding studies). This body weight loss approaches 30% after ~3 weeks and it has been concluded that single housing for adenine fed mice is not appropriate for long term studies (> 3-weeks). All mice will be extensively monitored daily and the severity of kidney damage will be assessed by urine and blood parameters.

It has also been reported that mice show an aversion to diets supplemented with adenine. Mixing the adenine in a casein-based chow effectively removes the inherent taste and smell of adenine. This has been found to work well in our studies so far with no evidence of high morbidity and mortality due to starvation and malnutrition rather than renal failure.

To avoid inconsistencies between diet batches these will be made commercially using powder diets which will continue to provide the adenine diets required. The diets will contain standard levels of calcium and phosphorous.

In some animal models rats rather than mice are more appropriate as pre-clinical models. Specifically:

- Ovariectomy is a routine procedure to mimic the menopause and is not expected to result in any abnormal symptoms. Both mice and rats can be used but rats are the preferred model as the bone loss more closely mimics the human situation and rats are the Food and Drug Administration (FDA) approved pre-clinical model for the evaluation of agents used in the prevention or treatment of post-menopausal osteoporosis. We have successfully used this procedure and no unexpected events were noted. However, any animal showing adverse effects that cannot be relieved by appropriate treatment will be killed by a Schedule 1 method. During observations of animals, particular attention will be paid to respiratory rate, colour of mucous membranes, levels of normal cage movement and grooming and feeding behaviour. Analgesics will be administered prior to the surgery to cover the immediate post-operative period.
- Pilot studies by us and others have shown that mice, unlike humans are not susceptible to poor bone health, hypoacidity and hypergastrinemia after prolonged administration of anti-acid treatments e.g. omeprazole which are commonly prescribed drugs to treat excess acid and "heart burn". This is likely because mice metabolise



these drugs differently from humans and therefore makes mice a poor model to translate the findings of animal studies to humans. In contrast rats dosed at 400 $\mu\text{mol/Kg/day}$ omeprazole show evidence of hypoacidity and hypergastrinemia.

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

To make the data obtained from animal experiments relevant to humans, the species of choice must be alive and have a similar physiology and skeletal system to humans. For these reasons rodents (rats and mice) are the standard animal model for studies of this kind as they are considered less sentient than non-human primates for example. Also mice are the standard species for genetic modification. Furthermore for my studies, it is important to study juvenile animals when the growth plate is fully formed and functional to mimic the growth period in children whereas in other studies it is essential to study adult rodents which have a fully mature skeleton resembling those found in our ageing human population.

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

Many of our protocols have been refined over the years and examples of these have been incorporated into the answer to the first section in this "Refinement" section. These refinements have resulted in procedures that have minimised pain, stress and suffering. Examples of further refinement include

- In certain methods e.g. adenine induced CKD we have also increased monitoring which involves the recording of body weight twice daily. A health score sheet will also be used from 3 weeks for mice on the CKD diet to determine humane endpoints.
- We noted on occasion that some rats injected subcutaneously (under the skin) with a substance (gastrin receptor inhibitor) developed ulcerated moist wounds which was an unexpected adverse effect. This was likely been compounded by daily scruffing for oral gavage for the delivery of another compound. To minimise this risk in future studies we refined our procedure by using an alternative placement of the injection (i.e. lower down and switching sides with each injection) to avoid scruffing in the area of the injection site. In severe cases rats will be euthanized using a Schedule 1 method but in milder cases they will be treated for minor inflammation and redness to around the area of injection on the head and neck.

We will continue to refine our procedures when unexpected animal welfare concerns are identified so that pain and suffering is kept at a minimum.

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

We stay up to date on best practice guidelines and updates by making use of the NC3Rs website (www.nc3rs.org.uk)

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



We will make use of the online resources provided by NC3R and follow developments in the literature. We will also keep informed on new developments with the help of informational seminars and events held locally (often brought to our attention via our Home Office Liaison Contact and Named Information Officer), and where applicable change our practice accordingly.

A retrospective assessment of refinement will be due by 25 March 2030

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?

27. The Metabolism and Kinetics of Chemicals

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

Pharmacokinetics, Metabolism, ADME, Chemicals, Rodents

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant
Rats	neonate, juvenile, adult, pregnant
Rabbits	neonate, juvenile, adult, pregnant
Beagles	, 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.

- 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 aim of this project is to perform metabolism and pharmacokinetic studies to support the development of non-pharmaceutical compounds (plant protection products (PPPs), biocides, industrial and household chemical substances, or novel foods, food additives, food enzymes and food flavourings). These tests, commonly referred to as ADME studies, are designed to investigate the Absorption of test substance related material (how substances get into the blood), Distribution around the body, (including into blood and urine), pathways of Metabolism (how the body breaks substances down), and routes and



rates of Excretion (how the body removes the substance), by harvesting samples (blood, plasma and other bodily fluids, tissues and excreta) at different times after administration. These studies will be performed for regulatory purposes to satisfy Global Safety Authorities that these substances are safe for humans if ingested deliberately (e.g. food flavourings) or exposed to them accidentally (e.g. Industrial Chemicals and Agrochemicals). They may also be non-regulatory studies performed to support these regulatory packages.

A retrospective assessment of these aims will be due by 20 March 2030

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 primary benefit of work carried out under this licence will be to allow regulatory authorities (who are totally independent from the commercial interests behind every marketing application) to come to decisions regarding the marketing of new compounds based upon safety data generated in these studies relating to exposure (pharmacokinetics) and ADME (metabolism) characteristics of the test compound. This will provide data supporting the rate and extent of absorption; distribution into tissues; key enzymes involved in metabolism; and the rates and routes of elimination; to assist in scaling safety margins of exposure and toxicity into man.

New agricultural chemicals may allow improved control of pests of food crops or of disease-bearing organisms. Testing of other chemicals may be necessary for new products that are designed to improve the quality of life. In all cases, the introduction of a new chemical may permit the removal of an older, more dangerous alternative, establish a new therapeutic approach or make an effective chemical more widely available, e.g. by reducing manufacturing costs. Testing of substances added to foods will ensure they are safe for human use.

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

The project will provide high quality, peer reviewed data for regulatory authorities, that will allow governments to decide whether the materials tested are safe for the public to use (Chemicals).

For Industrial Chemicals and Plant Protection Products, and other chemicals where there has been a regulatory call for data, there is an expectation, both public and governmental,



that materials to which people/animals/environment are exposed should be safe to use or that their potential hazards are well understood and documented. This is a regulatory requirement, and testing of such materials will be performed to satisfy the needs of global regulatory authorities under EC and OECD guidelines (including REACH, the Registration, Evaluation, Authorisation and restriction of CHemicals).

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

The primary benefit of work carried out under this licence will be to allow regulatory authorities (who are totally independent from the commercial interests behind every marketing application) to come to informed decisions, based upon data generated in these studies, regarding the risks and/or benefits when humans are exposed to chemicals.

The public will benefit from this data as will Sponsors, by obtaining data which allows them to make decisions on the development of their chemicals and to support regulatory filings.

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

The work will be shared with customers who will use it to determine their future strategy, or for submission in documents required by regulatory authorities. Whilst we have no direct control over what happens to the data after we have shared it, we trust from information given to us that it is used to support candidate selection or to support drugs progressing to clinical trials). Where appropriate, we collaborate with our customers to share data we have produced in the form of scientific publications that are in the public domain.

We are able to advise our customers on which studies are required in their development programme and on suitable study designs, based on our experience and on knowledge gained from previous feedback from customers and/or regulators, leading to focused and effective studies.

It is difficult to predict how the benefits of any work done on this project will be seen in the future due to confidentiality issues. However, this work will contribute to the pharmacokinetic/metabolic profiling and safety of chemicals that humans and animals are exposed to, by informing on safety

Species and numbers of animals expected to be used

- Mice: 5200
- Rats: 11350
- Rabbits: 590
- Beagles: 1040



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.

Rats, mice, rabbits and dogs may be used on this project. The majority of this work will be carried out in adult animals, however it may be a requirement to use juvenile or neonate animals to support pharmacokinetic/ADME work for drugs intended for non-adult populations.

Most studies on this project will be done in rats and mice as rodents are the lowest sentient species that have the physiology specifically similar to humans that meaningful predictions of human ADME, pharmacokinetics and pharmacodynamics can be made. Scientific opinion, including that of the regulatory agencies (who are independent of governments), indicates the use of one rodent and one non rodent species for many of the metabolism and pharmacokinetic studies that are required, to allow chemicals to be properly assessed for safety in humans. The rabbit maybe used when either previous work on a compound has started in the rabbit, or when the use of a rat or mouse is unsuitable due to pharmacological/physiological reasons, for example.

The most appropriate non-rodent species (dog on this licence) will be selected with reference to studies performed in rodents, evaluating cross-species metabolism and toxicity studies in test tubes (similarities in effects between rodents and dogs), and pilot pharmacokinetic and safety assessment studies. The species selected based upon this information is the one which is predicted to be most similar with human in terms of sensitivity, pharmacokinetics and metabolism. The minipig is considered the primary non-rodent species (these studies are conducted under a different project licence) unless there is specific scientific justification for the use of dogs or rabbits. Dogs are only used where the purpose of the programme of work can only be achieved by their use. Juvenile dogs would only be used for specific studies with suitable justification. No neonate dogs will be used.

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

Most of the animal procedures undertaken under this licence would be at a mild severity.

For a standard design, animals will be administered with a test substance (typically oral gavage or intravenous administration) followed by routine collection of blood and/or excreta. Following



completion of the sample collection, animals may either be killed by an appropriate method (to allow for tissues or carcass collection) or the animal may be returned to stock for re-use following a suitable veterinary examination (typically dogs).

Specific procedures may require confinement or restraint, but this is minimised wherever possible. Excreta collection generally required single housing to meet the objectives of the study, but this again is kept to the minimum period required to meet the study objectives (typically 1 week). Where possible, animals will be habituated to unfamiliar equipment or procedures to minimise stress.

Routine collections of blood will generally be taken by venepuncture of a peripheral/superficial vessel though implanted cannula may also be utilised where appropriate.

Studies vary in duration from a single dose to daily dosing for up to 4 weeks, depending on the intended use or likely exposure to each substance under study. Animals may be subjected to a series of linked regulatory procedures when studying a single test compound, such as cross-over design experiments. In this study type, or series of studies, animals may receive a dose of the test material by one route and after a suitable wash out period, a second dose, either at a different concentration or via a different route. Additionally where multiple compounds need to be tested, each with a similar mode of action or therapeutic theme, these may be administered in a series of experiments. Most dosing periods, will however, be short term ones (< 4 weeks).

Surgical models (typically biliary and vascular cannulation) are used to allow investigation into biliary excretion, to infuse a dose over an extended period of time, to replace biofluids or to collect serial samples (or any combination of these). For each surgical procedure, appropriate analgesia and balanced anaesthesia will be provided as standard and thereafter as required in accordance with a regime agreed in advance with the NVS. Animals will be under the care of highly trained technicians who will apply a rigid framework of welfare assessment to allow early detection of animals showing signs of discomfort or distress.

Some, less routinely used study designs, require the collection of tissues under anaesthesia. In these designs, the animal will be killed, under anaesthesia and not be allowed to recover consciousness.

Most rodents will be humanely killed at the end of procedures. However where the study type allows it, (such as blood sampling for pharmacokinetics or metabolic assessment) and subject to a suitable veterinary examination, animals (typically dogs) may be re-used in future studies.

If an animal is suitable for rehoming as a companion animal, after being considered suitable after a veterinary examination, this may also be allowed.



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

When dosing an animal by injection or taking blood, the amount of pain an animal feels is similar to what a patient would feel having an injection done, or having a blood sample taken by a doctor. If we have to repeatedly inject animals using a needle and syringe, we would choose different sites to do this where possible. If we can take blood samples when an animal is unconscious then we do. If we need to take repeated blood samples or need to dose repeatedly then we try and use different sites. Of course everyone who performs these procedures are trained to a high standard.

Animals undergoing surgery receive the same sort of care as a patient would in hospital. We discuss their pain relief and use of antibiotics with a vet before we start. We administer drugs as necessary and give them plenty of time to recover from surgery before we use them in experiments.

We often need to take a urine/fecal sample for analysis, so we would then put an animal into a special metabolism cage which requires the animal to be single housed. Virtually every animal will get used to their new cage within about 15 minutes and show no signs of distress.

Dosing with chemicals may cause adverse effects in some studies, although this is rare. We do observe our animals at least twice a day, and the people who do this know the signs when an animal is ill. If an animal is ill, we would check it more frequently, and get more senior staff involved in its care for advice, including vets. We also help sick animals out by giving more bedding, more heat and special food to make them more comfortable.

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)?

Under the last project licence, approximately 89% of animals experienced mild severity, and 11% of animals experienced moderate severity (animals mainly used after surgical preparation).

We would expect similar severities in this licence, however this would be dependent on the nature of the work performed.

What will happen to animals used in this project?

- Killed
- Kept alive at the establishment for non-regulated purposes or possible reuse
- Rehomed



A retrospective assessment of these predicted harms will be due by 20 March 2030

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?

Metabolism and pharmacokinetics are very complicated processes, and although there are metabolic tests that can be performed in-vitro (without the use of animals), there are no adequate models to replace the whole animal experimental model, as the complexity of the human body cannot be fully replicated in a test tube.

Similar international guidelines and directives are in place regarding agrochemicals and their ability to contaminate the food chain and industrial chemicals and their effect on the health and welfare of humans (especially if accidentally ingested).

Work conducted under this project will largely be for regulatory purposes, and this work is legally required by Global Regulatory Authorities. There are no accepted non animal tests that will allow the fulfilment of regulatory criteria alone, hence the use of animals is required.

Investigations are continually evolving into pharmacokinetic modelling from in-vitro data, thereby allowing better prediction of human responses and reducing the number of animals needed to generate the required data, but currently these remain limited in their reliability to go beyond indicating trends, due to the nature of complex metabolic processes in-vivo.

In many cases the protocols listed in this Project will be used later in the life cycle of a test substance and in many cases, in-vitro tests will have been conducted previously (often by the Sponsors) to ensure the drugs do what they are meant to, before we expose them to testing in animals.

Where non animal alternatives can be used and are accepted by regulatory authorities, they will be.

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

There are some studies that can be carried out in-vitro that can be used to support ADME work including tests that assess metabolism and absorption of substances, and how well they bind to key proteins in the blood. Predictive software can also be used.



Why were they not suitable?

None of these tests can yet model the complex and integrated mechanisms governing the ADME of chemicals fully and hence, animal testing is still a requirement. Work is on-going to refine the prediction of human pharmacokinetics from in-vitro data, although this is not currently at a stage where it can replace the animal models.

A retrospective assessment of replacement will be due by 20 March 2030

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 used on this project is estimated from those used under previous projects and after consideration of regulatory trends, and in review of future services being developed and offered for the screening and selection of new molecules for regulatory development studies.

Studies will be designed under this licence such that the minimum number of animals will be used in order to obtain the maximum information, whilst the scientific objectives of each study are met, in accordance with regulatory requirements and agreed standard practices.

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

We have a wealth of experience and knowledge regarding regulatory requirements and the industry standards used, hence we are in an ideal situation to influence study designs when discussing study requirements, and will always consider ways of reducing animal numbers. We also have available professionally trained statisticians to help design studies, if required

In order to fulfil the requirements of the regulatory agencies, animal group sizes for metabolism studies are variable, depending on the test substance being used, the specific type of study being undertaken and the strain of animals being used.



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

All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals. For study types that are less well established and for which historical data may not be available, the literature (scientific publications) will normally be consulted to help decide the group size. We can also consult with our statisticians, particularly where the study type is not routine and/or complex, as they can use calculations to estimate the correct number of animals needed to meet the objectives of the study

For studies where a new substance 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 levels we chose are safe, and the substance affects the system its designed to, without making an animal ill. These are called pilot studies.

We will try and get as many outputs as we can from a single animal where possible, without adversely affecting its welfare or compromise the main objective of the study.

Where possible, common control groups are used in order to minimise the numbers of animals used, or control animals are not used at all, where the doing so would not benefit the study design.

The re-use of animals, (see above) under carefully controlled conditions, under the supervision of a veterinary surgeon, also means the overall number of animals we use is reduced.

A retrospective assessment of reduction will be due by 20 March 2030

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.



Regulatory evaluation of chemicals generally requires investigation in two relevant mammalian species; usually one rodent and one non-rodent. This project predominately uses rats and mice as rodent test species.

For the non-rodent species are required, rabbits and dogs are used in this project. Selection of which is most suitable is considered according to the physical, physiological and behavioural requirements of the study, biochemical or metabolic similarities with target species (such as man), similarities of action and response to the compound of study, temperament and robustness in response to blood sampling.

The rabbit shares many characteristics with rodents but are considered a non-rodent choice when it comes to selection of a second test species to meet regulatory requirements. The minipig is considered the primary non-rodent species (these studies are conducted under a different project licence) unless there is specific scientific justification for the use of dogs or rabbits

All animals are monitored for signs of any adverse effects on their health or wellbeing, and to prevent unnecessary suffering, early humane end-points are applied under appropriate veterinary guidance (such as modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).

Highly trained staff use a rigid framework of welfare assessment to allow early detection of animals showing signs of discomfort or distress. We use pain relief as standard with anaesthesia and after procedures where relevant such as surgically implanted models. We sometimes, although rarely use animals that have had their genetic material altered such as such that they are predisposed to developing a condition or if a particular gene is important say in a specific component of the ADME process in which we have interest in.

Good Surgical Practice, including aseptic technique (Laboratory Animal Science Association 2017 Guidelines) will be observed for any animal undergoing surgical procedures, as well as the provision of pain relief and antibiotics, when needed. Environmental enrichments appropriate to the species are used within the animal facilities, such as play areas/toys, chew-items etc and except where the scientific objectives of a procedure or animal welfare considerations prevent it, all animals are group housed, so that they can interact and behave socially wherever possible.

Study designs are reviewed, and new methods considered as technology best practice and standards improve and advances become adopted and approved by international regulatory agencies.

Wherever possible, experimental samples are collected under anaesthesia or post mortem to minimise any potential suffering. In some circumstances safety markers will also be collected from the animals maximizing the data from individual studies. Maximising data decreases use of further animals and collecting samples post mortem or from terminally anaesthetised animals, minimises suffering.



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

Rats and mice will be used in most of the studies conducted under this licence. Rodents are considered to be the species with a similar enough brain/nervous system and physiology that will allow us to achieve the study aims and are considered suitable for the predicting what's likely to happen in humans.

For other species, there would need to be specific scientific justification provided for them to be used. This may be, for example, that other studies investigating a particular test substance have already been carried out in that species, or there is a particular metabolic pathway that is only available in that species, or say that is the target species for the actual test substance.

The use of dogs or rabbits will be driven by the need to fulfil regulatory requirements and to provide data to support the collection of safety data for substances humans are exposed to.

Some of the studies carried out under this licence will be some way into a development project where the scientific justification for the use of dog may have already been established. In that case the justification for the use of the dog under this licence will still be assessed using the information available.

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

Many of the procedures used in this licence are standardised, well defined and already well refined over many years. We will continue to assess any future possible refinements over the duration of this licence.

Where animals do show adverse clinical signs after dosing or surgery, we will increase the frequency and length of observations, and provide supplementary interventions (like extra bedding/food/heat) where needed, until the signs resolve.

Similarly, if after discussing with a vet and senior technician, we decide an animal is not recovering from procedures, and there is no prospect of them doing so in the near future, we will humanely kill them to prevent further suffering.

Habituation of animals to equipment like dosing tube and metabolism cages will be carried out prior to the start of the dosing phase.

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

Regulatory guidelines

COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of



the European Parliament and of the Council concerning the placing of plant protection products on the market.

The testing of plant protection agents or biocides will be performed to satisfy European and global regulations; US EPA OPPTS 860.1300, OECD 417, 503, EU 7030/VI/95 and 98/8/EC with subsequent amendments.

Industrial chemicals require testing under the procedures for the Registration, Evaluation, Authorisation and Restriction of Chemicals [REACH] within the EU (OJEU L 396, 30 December 2006).

The investigation of chemicals and substances that are added to foods intended for consumption by man or by animals is the subject of multiple European Directives, often with subsequent amendments. Examples include Council Directives 89/107/EEC on food additives intended for human consumption (OJEC L40, 11 February 1989), 70/524/EEC concerning additives in feeding stuffs (OJEC L270, 14 December 1970), 88/388/EEC on flavourings for use in foodstuffs (OJEC L184, 15 July 1988), 89/398 on foods for particular nutritional uses (OJEC L186, 30 June 1989) and 2002/46/EC on food supplements (OJEC L183 12 July 2002).

OECD Guidelines for the Testing of Chemicals Section 4: OECD 417; Toxicokinetics (2010).

DIRECTIVE 98/8/EC (16 February 1998), and Regulation (EU) No 528/2012 (22 May 2012 and subsequent amendments); concerning the placing of biocidal products on the market.

Nohsan No. 8147 (2000): The guidelines related to the study reports for the registration application of pesticide Appendix to Director General Notification, No. 12-Nousan-8147, 24 November, 2000, Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan.

Surgical Procedures

LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017)

Blood sampling and dosing

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).

Diehl et al (2001). A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23.

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

We have a dedicated regulatory affairs team that monitor regulatory changes and feed those back to the scientists who do this work.

A retrospective assessment of refinement will be due by 20 March 2030

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. Modulating inflammation and immunity

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

Immune homeostasis, Infection and immunity, Inflammatory bowel disease, Cancer, Lipid mediator

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.

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 overall aim of this project is to examine how fat molecules regulate the immune system (especially at the barriers such as gut) and their impacts on diseases such as inflammatory bowel disease and bowel cancer.

A retrospective assessment of these aims will be due by 25 March 2030



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?

Immune cells control epithelial homeostasis and inflammation, and the immune system in turn is sensitively regulated by a system of soluble factors including fat molecules (scientifically called lipids). A dynamic balance of harmful and anti-inflammatory factors, for example proteins and lipids produced by different cells in the body, is required for safeguarding the barrier integrity in healthy individuals.

Disruption of this balance results to uncontrolled, excessive harmful responses, in turn leading to the development of inflammatory disorders like allergy (e.g. food allergy), inflammatory bowel disease (IBD), and cancer. Emergent research is thus desired for better understanding of how barrier tissue health is maintained by these soluble factors, especially fat molecules, in the healthy organ.

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

The benefits of this research will be to provide novel understandings of mechanisms for the development of (epithelial) inflammatory diseases such as asthma, atopic dermatitis, IBD and bowel cancer, to identify new therapeutic strategies for controlling these diseases and for avoiding drug adverse effects, especially NSAIDs), therefore to improve human health and life quality.

This project will advance the field by defining mechanisms for how epithelial inflammation is developed, expand our understanding on the role of the lipid-immune crosstalk in the setting of inflammation in the lung and gut. It will provide new findings on why NSAIDs can treat inflammation and cancer and why these drugs can also have adverse effects on intestine and lung.

Main outputs will be research reports, publications including peer-reviewed research articles and non- peer-reviewed presentations (oral and/or poster). The research will also define new drug targets and thus link to development of new therapies.

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



The beneficiaries include clinicians, patients, pharmacies, academic researchers in related fields as well as the public. We will present and discuss our results in the research community through direct meetings, seminars and workshops. We will also transfer new information and understanding generated from our research to the next generation of researchers (post-doctoral fellows, post-graduate and undergraduate students) through workshops for training or dissemination of information. We have close communications with clinicians which enable exchange of knowledge of inflammatory diseases with clinicians. This interaction will ultimately improve the quality of medical treatment for human and other animals (i.e., veterinary clinical patients). The data derived from this project will be also beneficial for drug discovery programmes and are therefore of interest to the pharmaceutical industry. Government departments linked to biomedical research and public health and charitable bodies such as NHS will be also benefitted from this research. The results of this research will also be disseminated broadly to the public through the news agencies or direct public engagement events.

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

We will collaborate with various groups including academic colleagues and clinical doctors, disseminate our new findings to patients and the public via public talks (e.g., Pint of Science, Science Festivals, patients events). We will also try to publish negative results and unsuccessful approaches when appropriate.

Species and numbers of animals expected to be used

- Mice: 7,100

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.

This project involves various model systems of disease including inflammation and cancer, which are highly complex processes involving cell-cell, cell-matrix and host-microorganism interactions along with accumulation of multiple genetic and epigenetic abnormalities in a consecutive manner. For example, development of asthma or IBD depends on damages on epithelial cells, activation and differentiation of innate and adaptive immune cells, and altered structure of stromal cells (like matrix) and microorganism communities in the lung and gut. Therefore, it is impossible to reconstruct this process in a single in vitro system. Furthermore, studies on new therapies and treatment of established disease cannot be conducted solely on in vitro cell or organoid cultures because the development of disease is so complex that involve multiple organs in vivo.

For most studies proposed in this project will be carried out on young adult animals (e.g., >7 weeks old mice), but mice at different life stages may be used according to the nature



of the research and diseases. For example, to study immune mechanisms for food allergy in very young children, neonate mice will be used as they have different type 2 immune responses compared to adults. In order to study the effects of age on immune functions and cancer (given that age is an important risk factor for cancer and infectious diseases), aged mice (>12 months old) will be used.

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

A typical experiment will involve inducing immune activation, gut injury or cancer development induced by administering reagents or cells through diets, drinking water, oral gavage, intraperitoneal, intravenous, or subcutaneous injections, or topical or rectal applications, followed by modulation of immune responses.

The most invasive protocol would in addition to the steps above also include modulation of transgene expression, modulation of blood stem cells, and modification of diet or gut microorganisms. The animals will be generally housed in groups in a cage, but individual housing may be used when needed, for example to examine the effect of gut microorganisms.

To study the impact of stress on immune function, health and drug efficacy, animals may be restrained in a specific device (e.g., in a tube).

Typically, animals will experience mild, transient pain and no lasting harm from administration of substances by injection using standard routes (intravenous, subcutaneous, intraperitoneal). Where administration is required for prolonged periods, animals will be surgically implanted with slow release devices such as a mini-pump. These animals will experience some discomfort after surgery and some mild to moderate pain which will be treated with analgesics. Animals will undergo changes in diet which are not expected to cause distress but may sometimes result in obesity or itchy skin. Some diets may result in weight loss due to unpalatability - if this is the case non-caloric sweetener powder will be added to the food. Animals will be placed onto normal diet should they lose 20% of their body weight.

Animals will experience mild and transient discomfort from blood sampling. The final procedures will be undertaken under non-recovery anaesthesia where the animals will only be aware of the anaesthetic being administered and may experience mild distress and no pain.

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

Infection is generally well tolerated by rodents without anticipated mortality or systemic pathology. Mortality in animals subject only parasite infection is not expected to exceed 1% and under conditions used are not expected to be procedure-related. Infection models in this project are widely used laboratory models which are tolerated at the doses used. Co infections may promote propagation and recovery of one or both species involved.



The use of immunological booster ingredients may lead to formation of small lump of the tissue affected and recurrent or persistent ulceration with accompanying pain. The repeated use of antigens in the immunological booster ingredient alum can lead to severe allergic reaction. The general use of immunological booster ingredients and targeted delivery of plasmids may cause local tissue redness and pain and/or skin lesions, with the possibility of ulceration which may demand culling by a Schedule 1 method. The incidence of such effects is expected to be below 1% for the duration of this project.

Gut inflammation (e.g., colitis) and some cancers (e.g., bowel cancer) is associated with body weight loss and clinical signs such as blood in faeces and diarrhoea. Animals may lose 20% of body weight and then begin to gain weight. Rectal prolapse is not commonly seen. Altered general appearance (hair standup, lethargy) occurs in the majority of animals. In acute colitis models (e.g., the DSS model), abdominal pain, diarrheal, bleeding, hair standup and hunched posture occur in the majority of animals and last for a few days. In chronic colitis models (e.g., naïve T cell transfer model), mice mainly have weight loss and mild diarrhoea for weeks, but rarely have pain, bleeding, hair standup and hunched posture.

Mice with cancer development can show mild symptoms (such as mild hunching and hair standup in ~20% of mice), weight loss, anaemia, diarrhoea, and rectal bleeding. Animals will be monitored closely and frequently, and animals will be terminated when they are found having weight loss of 20%, subdued behaviour, anaemia (as shown through paling of the feet), sustained rectal bleeding with visible blood on fur, and sustained diarrhoea for over 72 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 animal type)?

Protocol 1: Breeding and maintenance of GA mice: 90% sub-threshold, 10% mild.

Protocol 2: Gene expression: mice, 70% mild, 30% moderate.

Protocol 3: Immune homeostasis, infection and immunity: mice, 60% mild, 40% moderate.

Protocol 4: Gut inflammation: mice, 10% mild, 20% moderate, 70% severe.

Protocol 5: Cancer: mice, 10% mild, 20% moderate, 70% severe.

What will happen to animals used in this project?

- Killed

A retrospective assessment of these predicted harms will be due by 25 March 2030

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 involves a range of model systems of pathology including inflammation and cancer, which are highly complex processes involving cell-cell, cell-matrix, and host-microbiota interactions along with accumulation of multiple genetic and epigenetic abnormalities in a sequential manner. For example, development of IBD depends on damages on epithelial cells, activation and differentiation of innate and adaptive immune cells, and altered structure of stromal cells (like matrix) and the gut microbiota. So that it is impossible to reconstruct this process in a single in vitro system. Furthermore, a major clinical need is for new therapies and treatment of established disease. As the development of disease is so complex, studies on new therapies and assessment of their side-effects must be conducted on in vivo models.

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

We use every effort to try in vitro assays before going to in vivo animal models. For example, we have used in vitro immune cell culture system to assess the effects of lipid mediators on activation, proliferation, differentiation and cytokine production of single immune cell type (e.g., T cells). We have also carried out analysis of human biopsy samples to measure lipid levels as well as bioinformatics analysis and calculated their correlations with immune cell activation. We also considered to set up organoid culture (e.g., intestine tissue cells) or culture of mixed multiple cell types (e.g., T cells together with antigen-presenting cells like DCs or macrophages) to mimic interactions between immune cells and non-immune cells, e.g., T cell-DC coculture for mimicking antigen presentation and T cell activation.

Why were they not suitable?

The above in vitro culture systems provide essential information on immune cell behaviour and functions in response to external stimuli. As described above, however, the complexity of interactions among different cell types in addition to host-microbiota interactions in vivo cannot be fully mimicked by in vitro cell cultures and computing simulations.

A retrospective assessment of replacement will be due by 25 March 2030

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 did calculations using typical variations from our own earlier experimentation to calculate minimum numbers of animals to be used whilst ensuring that the results are statistically significant. Sample sizes for our experiments are estimated from past experiments. Calculations typically show that we need group sizes of 10-12 to achieve the quality of results we need. We will pool data from independent repeat experiments to maximise the efficiency of animal use. We have used our annual return of procedures data to estimate the number of animals that we will need to use for breeding.

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

We employed the NC3Rs' experimental design guidance and experimental design assistant (EDA) to plan our experimental design, practical steps and statistical analysis utilising the advice and support for randomisation and blinding, sample size calculations and appropriate statistical analysis methods. We will use the EDA diagram and report outputs to support experimental planning with animal users.

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

To reducing the variability of data and thus helping to reduce the number of animals required, particularly relating to intestinal inflammation and tumour formation, we will carry out most, preferably all, of related work using IVC cages (according to their availability in the facility) to protect their health status. We will also use both in vitro studies and small pilot experiments prior to determining the final experimental design. All mouse lines may be cryopreserved and held in archives. This will allow us to terminate to breed the lines of mice when the experiments on these mice have completed, so that reduce the maintaining mice numbers. At the end of the experiment, we will harvest as many tissues as possible at post-mortem. If we don't need to analyse the tissues immediately, we will freeze them and make them available to other researchers working on similar questions.

A retrospective assessment of reduction will be due by 25 March 2030

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.

Some animals will be allowed to grow old to study the immune response in the ageing animal. To induce gene expression in animals or to deplete specific cells, some animals will be given substances by mouth, injection, or through food. Oral gavage or injection can be necessary to induce a rapid change in gene expression. This will let us to study processes that happen within short time periods of a few hours. We will have to induce immune responses to study the response to infection or treatments. Animals will be treated using methods similar to human vaccination, e.g., injection of substances under the skin, into the peritoneal cavity or by intravenous injection. To number animals for group housing and faithful genotyping of transgenic animals,

The model systems entailed in this Project are selected as the most appropriate parallel to human disease conditions while sufficiently refined to minimise impact on the individual animals. Severe outcomes may also occur in models of inflammatory bowel disease and bowel cancer. These models are highly reproducible with a predictable course in standard strains of mice with well-defined protocols the course of disease is predictable although less is known about the contribution of different immune cell types. Because of this, different models allow testing of the innate and adaptive immune populations, and their interaction, in a complex disease involving intestinal microbes as well as the immune system itself. Due to this complexity, an in vivo model is the only means available to test the efficacy of new interventions and potential cures for human disease.

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

Mice are chosen for all work planned in this Project, as they are sufficiently close in anatomy, physiology, genomics and pathology to humans, thus offering a well-defined host species with many well-characterised models for a range of serious human diseases. The availability of genetically altered mouse strains and reagents (e.g. antibodies to surface markers) provides an unrivalled access to experimental manipulation at the most refined level currently possible.



Non-mammalian animals are limited in their use because they either do not have the right type of immune cell or their immune system is too different from the human immune system to provide relevant results. We can't use embryos as their immune system is immature and doesn't respond to antigenic stimulation in the way young or mature animals do.

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

Ageing animals will be carefully monitored by staff trained to work with ageing animals. Group sizes in ageing experiments will be increased to accommodate for loss of animals and to avoid single housing due to animal losses due to old age. Longer drinking spouts will be used, and animals will be monitored for adverse effects such as changes in weight, dermatitis, piloerection, paleness, changes in mobility, lumps, eye defects, abnormal respiration, or stools. If these are observed, animals will be treated accordingly and immediately humanely killed when they reach the humane endpoint.

We will continue to use the least invasive and distressing procedures available to reduce harm on the animals such as pain, suffering, distress. For example, we will carefully select the most appropriate route to administer reagents to mice.

To avoid long-term, repeated injections, osmotic pumps will be used to provide convenient, reliable, and cost-effective alternative to chronic injections, to ensure constant compound levels in plasma or tissues for maximized therapeutic efficacy and reduced adverse effects, and eliminate the need for frequent animal handling. This will not only make animal more conformable but may also reduce animal numbers potentially because the effects of reagents will be optimised.

Animal suffering will be minimised. The animals under study will be closely monitored on a daily basis to ensure relevant disease progression with the minimum harm to the animal. Disease scoring system will be used form certain models (such as colitis). If mice are found to reach the limited score or severity, they will be immediately terminated. For cancer models, the work will be undertaken in accordance with the principles set out in the Guidelines for the Welfare and Use of Animals in Cancer Research: British Journal of Cancer (2010) 102, 1555-1557 (i.e., NCRI Guidelines) and in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010).

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

We will follow published guidelines to assist with planning animal research and testing, such as the PREPARE guidelines and other publications from the NC3Rs and Laboratory Animal Science Association.



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

We will regularly check information on NC3Rs website, we've signed up to the NC3Rs newsletter, we will meet the NC3Rs Regional Programme Manager, and attend Regional 3Rs symposia.

A retrospective assessment of refinement will be due by 25 March 2030

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. Inflammation in cardiovascular 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

cardiovascular disease, heart failure, inflammation, therapy

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

Past studies have shown that the cell skeletal structures are involved in regulating cardiovascular disease (CVD) progression through mediating the inflammatory response. To identify and validate the potential relevant therapeutic targets, we will investigate the role of associated mechanisms in inflammation, particularly in the setting of CVD.

A retrospective assessment of these aims will be due by 23 February 2030

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?

CVDs are the leading cause of death globally. CVDs cause around 1 in 3 deaths globally, an average of 56,000 people each day or one death every 1.5 seconds. Within CVDs, heart failure (HF) is a global pandemic affecting at least 26 million people worldwide, and the number is likely to increase due to an ageing population. HF is a medical condition in which the heart has a reduced ability to pump blood from its chambers. Despite the availability of some therapies, the prognosis of patients with HF remains poor. Therefore, investigating the new mechanisms for regulating the disease development of CVDs and developing treatments to improve outcomes of patients with HF are in high demand. Previous studies have shown that cell skeletal structures are involved in regulating inflammatory output and the progression of CVDs including HF after heart attack. Further understanding the role of cell skeletal structures in regulating inflammation and cardiovascular cell function may help us to find treatment plans for relevant heart diseases.

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

The project will provide new knowledge in understanding the cell skeletal structures - associated roles in regulating inflammation and heart diseases.

Current treatments to increase heart pumping function in treating heart failure are deemed unsafe due to the increased death rate which is associated with treatments. The project may provide new treatment targets and new therapeutic strategies for improving heart function in treating heart failure.

Diastolic heart failure is a condition where the main pumping chamber of the heart (left ventricle) becomes stiff and unable to fill properly with blood during the relaxation phase of heart contraction, therefore affecting the amount of blood pumped out to the body. This type of HF is strongly associated with inflammation. The project will help us understand the roles of cell skeletal structures in this type of heart failure. It may provide new treatments for this disease.

The results of this project will be published in peer-reviewed journals and presented at relevant conferences. This project may lead to patent applications and provide important pre-clinical information for translation into humans.

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

There are around 7.6 million people living with CVDs and around 920,000 people living with HF in the UK. CVDs significantly reduce both the quality and duration of life, accounting for over 25% of all UK deaths – equivalent to 450 daily fatalities or one every three minutes. CVDs put a substantial social and economic burden on the UK (Data from BHF statistics compendium).

In the short term, the impact of our outputs will include:

The knowledge gained from our project will benefit a broad medical and scientific community including cardiology and beyond. Cell skeletons are structural networks within



the cells and play important roles in different cellular processes. The dependent mechanism in regulating inflammation could also be used in understanding other inflammatory diseases/conditions beyond inflammatory CVDs.

The medical community and pharmaceutical companies will benefit from our outputs to develop new treatments for relevant cardiovascular diseases and inflammatory diseases. The techniques further developed in this project (for instance different models of heart failure) may help other researchers in this field and therefore will refine or reduce mice usage in their future experiments.

In the long term, the impact of our outputs will include:

The novel treatments identified in this project may provide proof-of-concept evidence for future clinical trials and eventually provide safe and effective treatments for patients with heart failure and other inflammatory diseases.

The knowledge gained from this project may help provide detailed information to aid in the diagnosis of heart diseases with accuracy to match the relevant treatments.

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

Our previous and present projects are based on wide collaboration with different research groups worldwide. We aim to publish our results in scientific open-access journals with high impact. We will present our findings at international conferences and disseminate the new findings to the public. We'll also aim to find ways to communicate or publish unsuccessful results through conferences and openaccess platforms. Our group will use the Equity, Diversity, and Inclusion (EDI) approach to involve suitable researchers from different backgrounds in the projects to maximize the output.

Species and numbers of animals expected to be used

- Mice: 15300
- Rats: 300

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.

Our experiments will be conducted in rodents. We use the mouse model in our project for the following reasons: 1). Mice and humans share a very similar set of genes. On average, the protein-coding regions of the mouse and human genomes are 85% identical; some genes are nearly 99% identical. In addition, because of the high breeding rate, the mouse model is a useful genetic model to address human biological problems. 2). There are well-established methods for mouse handling procedures. 3). The mouse models of different cardiovascular diseases including myocardial infarction (heart attack), heart failure (heart doesn't pump enough blood for the body's needs), and atherosclerosis (blockage of arteries) have been well-established and widely validated.

Adult rats will be used in heart attack models to screen effective therapeutic strategies. In some cases, rats can be advantageous due to their larger blood volume and ease of administration, allowing for more accurate dosing and monitoring of drug effects. Because of their physiological similarities to humans and their longer lifespan, results from rat models are closely translatable to human diseases. This study will help to provide essential preclinical evidence before the human clinical trial.



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

In one disease model, the animal will undergo a surgical procedure that will cause heart vessel blockage. This will be induced through one-hour surgery by creating small chest windows to access the heart and blocking a coronary artery with a single stitch. After surgery, the animals undergo regular monitoring and non-invasive imaging under anaesthesia to track disease progression. Painkillers will be applied to these animals which undergo surgery to reduce discomfort and moderate to severe pain. They will be humanely sacrificed either at an early stage (within 3 days) or a late stage (3 to 6 months) under terminal anaesthesia (a state of induced unconsciousness) to minimize suffering. In another disease model, the animal will experience changes in food (high-fat food) and/or drinking water (chemicals inducing heart disease administered in water) lasting 5 to 15 weeks, expected to be non-distressing. Some animals may need to be housed individually in cages for short periods (48-96 hours) after receiving energy sources such as carbohydrates, insulin, and lipids. This allows for accurate measurement of food and water intake as well as urine and feces output. At the end of the experiment, animals will be humanely sacrificed under terminal anaesthesia.

Additional procedures may be included in some experiments. For example, administration of substances (e.g. treatment drug) by injection using common methods such as in the veins, under the skin, or in the abdominal cavity. Where administration is required for prolonged periods, animals will be surgically implanted with slow-release devices such as a mini-pump under anaesthesia. These animals will experience some discomfort after surgery and some mild to moderate pain which will be treated with painkillers. Some animals will experience mild and transient discomfort from blood sampling. In another situation, animals will be exposed to radiation and have their bone marrow replaced to study how the immune system affects diseases.

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

The possible adverse effects are listed as follows:

1 After the procedure that leads to reduced heart function, we expect to observe potential signs of heart failure in animals. These signs include a hunched posture, difficult breathing, decreased activity, possible immobility, signs of distress resembling a heart attack, and weight loss associated with heart failure.

These adverse effects primarily result from the development of these disease models. In most cases, they typically last for only a few hours. In certain instances, some effects may extend up to 48 hours to 1 week. All animals are subject to regular monitoring to proactively identify and address any adverse effects. This may involve pain management using pain relievers, immediate post-procedure fluid resuscitation with saline when necessary, or, if humane endpoints are approaching, a humane termination of the experiment.

A small percentage of animals (5-10%) may die during surgical procedures due to complications related to the procedure or the anaesthesia. Additionally, approximately 20-30% of animals may die due to life-threatening arrhythmias (abnormal heart rhythms) or cardiac rupture (tear in heart tissues) resembling sudden cardiac death in patients with heart conditions. These events are unpredictable and cannot be prevented in advance. Mice will have minor surgery to implant a device under the skin that can release a medicine slowly. They are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in a hospital.



Imaging will be performed on animals to monitor the progression of diseases. The procedure is conducted under anaesthesia (unconscious state). The animals are expected to regain full activity within 15 minutes after the procedure.

Animals receiving bone marrow transplants may experience a temporary period of illness lasting several days following irradiation. During this time, they may reduce their food intake and potentially lose weight, up to 10% of their pre-irradiation weight. However, we anticipate that they will begin to regain their body weight about 14 days post-irradiation. The health status of irradiated animals will be closely monitored through daily checks and at least twice-weekly weigh-ins.

Single housing can cause stress in mice, but we will minimize the duration if possible. Whenever feasible, we will reintroduce animals to group housing with their original cage mates after a period of single housing.

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)?

Mice: Mild (67.5%), Moderate (25.1%), Severe (7.4%)

Rat: Mild (5%), Moderate (65%), Severe (30%)

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at the establishment for non-regulated purposes or possible reuse

A retrospective assessment of these predicted harms will be due by 23 February 2030

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?

Currently, there are no non-animal alternatives that can be used to accurately predict or mimic the development of heart disease. Current heart cells used in culture are not mature, and they have limitations to be used to explain heart disease development such as heart attack which is a disease condition occurring in human adults. This is why we need to conduct experiments on animals to explore the fundamental mechanisms and identify potential ways to treat them.

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



We've explored the use of stem cell-derived heart muscle cells as a substitute for mature adult heart cells.

We're currently working on developing a method to grow mature heart muscle cells in a lab dish. This could potentially replace a lot of the experiments that currently use mice. Also, by growing these heart cells in certain conditions, like low oxygen, we might be able to create a model of heart damage without needing to do surgeries on animals, which can cause them discomfort.

Why were they not suitable?

Human-inducible pluripotent stem cell (ips)-derived heart muscle cells as a replacement in our study are not suitable. These cells are not mature cells and do not have mature cell skeletal alignments and associated mechanisms present. We're currently improving the method of the mature heart muscle cells in vitro culture.

A retrospective assessment of replacement will be due by 23 February 2030

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 estimated the number of animals majorly based on our previous data and experience, and results from pilot experiments. We also referred to published data from other research groups, and suggestions from our collaborators.

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

We used the free online tool from NC3Rs (Experimental Design Assistant) to design our experiments to ensure we used the minimum number of animals. We followed the PREPARE guidelines (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and the CAMARADES/NC3Rs systematic review facility recommendations for the design and analysis of our experiments.

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

We'll carry out a pilot experiment with a small number of animals in each disease model. According to the statistical analysis by integrating the acquired information from the pilot data and past studies, we will calculate the power (the number) of animals to be used.



Efficient breeding will be achieved by choosing the right mouse strain and providing a clean and controlled housing environment with proper temperature, humidity, and lighting. Male-female pairs will be introduced at the right breeding age. Detailed and accurate records of breeding pairs, births, and litters will be recorded to track the lineage and genetic information. Lighting cycles will be controlled to mimic natural day and night cycles as this can influence breeding behaviour. The pups will be weaned at the appropriate age (usually around 3-4 weeks) to prevent inbreeding and overcrowding. Genotyping (a method to examine the gene traits) will be timely performed to confirm the genetic characteristics of the mice. New breeding pairs from other sources will be periodically introduced to maintain genetic diversity. Imaging techniques (e.g. Echocardiography is a type of ultrasound scan to create pictures of the heart) will be used in our heart failure model to track the heart function non-invasively, so we can get more data from the same animal. At the end of the experiment, we will harvest as many tissues as possible. We will freeze the samples and make them available to other researchers working on similar questions if the spares are available. Freezing embryos and sperms will be used to maintain the genetic modification breeding with the least live mice.

A retrospective assessment of reduction will be due by 23 February 2030

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 primary goal of the current project license is to understand the mechanism that governs the progression of CVDs, with a particular focus on heart failure. We employ mice and rats as our animal models, as they have demonstrated high suitability for investigating these disease processes.

We'll use the left anterior descending coronary artery ligation to induce ischaemic heart failure (IHF, a type of heart failure type due to lack of blood supply). The left anterior descending coronary artery is the vessel that provides oxygen and nutrients to the heart. When it's tied off, the heart muscle doesn't get enough blood and oxygen, making the heart difficult in pump blood throughout the body. The artery ligation requires surgical procedures that will be conducted under general anesthesia and pain management for a comfortable recovery. Continuously, we closely monitor these animals for any signs of discomfort or distress, allowing us to halt the experiment if necessary to minimize any potential suffering. This enables us to terminate experiments based on humane endpoints, ultimately minimizing any suffering endured by the animals involved.

In another model, the animal will be induced to heart failure with preserved ejection fraction (HFpEF). Under this condition, a heart becomes stiff and unable to fill enough



blood as it should. The HFpEF model is not expected to cause more than mild distress. Currently, there are no other available heart failure models that involve less suffering.

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

Non-mammalian animals are limited in use due to significant differences in their cardiovascular systems compared to humans. Among mammals, mice and rats are the most useful species for gaining relevant and meaningful pathophysiological information. Mice also offer the advantage of genetic manipulations, enabling us to study the underlying mechanisms.

Embryos and very young animals are not suitable for our research because heart attack and heart failure are medical conditions occurring in adults.

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

During surgery, we will use the Rodent Surgical Monitor (equipment monitoring the vital signs, such as body temperature, heart rate, and breathing, in mice and rats) to continuously track the animal's health and ensure the animal's well-being. If any anaesthesia or surgical issues arise that may endanger the animal, we will immediately terminate the experiment and ensure humane euthanasia.

After surgeries, animals will be placed in individual recovery cages with food and water access. Mice will be re-grouped upon full recovery. Animal behaviour will be monitored hourly before they fully recover and at least twice during the first week after surgery to detect signs and distress at an early stage. Scoring sheets will be used to assess the welfare status of animals and establish early humane endpoints wherever possible. We'll use imaging to keep track of how the animals' diseases are changing, and they'll be given inhalation anaesthesia like isoflurane instead of injections like ketamine/xylazine. This should make it easier for them to fall asleep smoothly, adjust their sleepiness quickly, and wake up faster. It's also expected to be safer for their hearts and breathing, and they should handle it better overall.

We acclimate animals to changes in their environment as needed. For instance, when evaluating individual food intake and metabolic processes, animals may require solitary housing in specialized cages for up to 7 days. Single housing can sometimes induce stress, leading to potential aggressive behaviour when re-introduced to their littermates. To mitigate this, we'll follow recommendations as detailed on the NC3Rs website, utilizing pre-exposure to soiled bedding for several days before regrouping as a refinement measure to reduce aggression. Animals on a tamoxifen diet may experience weight loss (10-20%) in the first few days. To mitigate potential aversion to eating, sweeteners or other palatable substances will be incorporated when administering tamoxifen to animals via diet.

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

We adhere to best practices to refine our experiments, including guidance from the Experimental

Design Assistant (<https://eda.nc3rs.org.uk/>), internal guidance developed by the Named Veterinary Surgeons for tamoxifen use, recommendations for pilot studies (<https://www.nc3rs.org.uk/3rsresources/conducting-pilot-study>) in the NC3Rs resource library, the ARRIVE guidelines, PREPARE guidelines, and LASA Aseptic surgery guidance.



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

We will regularly monitor the NC3Rs website, subscribe to their newsletter, and participate in Regional 3Rs symposia. We've recently joined the NC3Rs cardiovascular network. We'll stay updated on the newest models for cardiovascular research, participate in network meetings, and offer feedback to help support the cardiovascular research community. We also adhere to the ARRIVE guidelines and stay updated on advancements in the 3Rs, such as those found on the Norecopa platform (<https://norecopa.no/alternatives/the-three-rs>) and the Danish 3R-Centre (<https://en.3rcenter.dk/3r/replacement>). Our goal is to swiftly integrate these advancements into our practices during the project.

A retrospective assessment of refinement will be due by 23 February 2030

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?