Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted July - September 2023 that require a retrospective assessment

Contents

1. Molecular neuroregeneration

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

Nerve injury, Spinal cord injury, Regeneration, Repair, Molecular mechanisms

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?

Nerve and spinal cord injuries are followed by regenerative failure and long-term disability that remain without treatment. This project aims to investigate the molecular mechanisms underpinning nervous system repair in nerve and spinal cord injuries by using rodent animal models that mimic the human condition and to test novel treatment strategies.

A retrospective assessment of these aims will be due by 13 January 2029

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 of the outmost importance and high priority because millions of people worldwide and two million in the UK alone suffer from nerve and spinal cord injuries that lead to long term neurological disability for which a cure remains elusive. The disabilities resulting from neurological injury affect patient autonomy for daily activities and increase the susceptibility to non-neurological illnesses, such cardiac, reproductive, psychological and immunological disorders, all of which contribute to a dramatic socioeconomical weight. So far treatment options have not been able to stimulate sufficient neuronal regrowth and reconnection that are essential for functional recovery.

A better understanding of the injury-related biological processes that stop neurons from regrowing can help identify new treatments that target and counteract those limiting mechanisms stimulating regeneration. Further work under this project license will also facilitate the development of those newly identified treatments increasing their clinical application. Regenerative treatments can help patients regain autonomy, motor and sensory function, and decrease the incidence of concomitant disorders, which would in turn decrease the impact of the injury on the care-takers life-style and finances. Since estimated average lifetime cost of health and social care for a person with a spinal cord injury in the UK is £1.12 million, decreased prevalence of chronic injuries and comorbidities will also have a beneficial impact on the NHS and related health care providers.

This project license will target the influence of, among other regeneration regulators, neuronal metabolism (controlling neuronal energy management), the interactions with the immune system, ageing, environmental factors and the circadian cycle on nerve and spinal cord regeneration after injury. The molecular mechanisms of these factors on neurological injuries are still largely unknown, and their characterization will have a tremendous scientific impact providing large amounts of data that will be useful to the scientific field for future therapeutic discovery.

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

Our work will result in standard scientific outputs similar to those generated across the duration of the previous license period. These include, publications relating to the individual aims of the work set out, invited talks and organised conferences to share relevant findings with colleagues and collaborators and patents, relating to developed interventions and the leveraging of additional funding to support ongoing work. In addition, the programme will generate a rich data set consisting of functional and structural longitudinal measurements from thousands of nervous system cells in animals after nerve and spinal cord injuries across the lifespan. The molecular signatures of these cells will also be correlated to behavioural measures collected from the same animals as far as their repair ability. These outputs, gene, protein and metabolite expression datasets, will be shared freely making them available to other researchers, computational neuroscientists

and clinicians interested in neurotrauma and neurodegeneration.

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

Today in the UK, more than 2 million people are affected by nerve and spinal trauma, over 20 million in the US for example. The most impactful benefits arising from this research programme include an improved understanding of the basic biological processes governing the capacity of axons to regrow in the nerves and spinal cord following nervous tissue injuries. Axons are the extension of the neuronal cells that physically connect the brain with the spinal cord and the rest of the body, and provide the exchange of information between them. This may lead to the development of new therapeutic modalities for these neurological diseases that could then be evaluated in clinical trials in patients affected by nerve and spinal cord injuries. Due to a lack of effective therapeutic options that limit long- term neurological impairment in these disorders, this work should be considered as of high priority. It may in fact lead to benefit for patients suffering as well as for social burden linked to the high costs of patient care.

Our research will lead to short, medium and long-term benefits. Specifically, we expect to achieve the following potentially beneficial goals:

Short-term (2-3 years)

1. Clarification of the systemic and molecular basis for the capacity of neurons to regenerate in animal models of nerve and spinal injury, with special emphasis on spinal cord trauma, sciatic nerve injury and optic nerve crush. The benefit will be in the elucidation of factors that may limit regeneration and repair. This benefit will specifically be achieved using a combination of techniques (molecular biology/electrophysiology/imaging/behaviour) that allow direct measurements of molecular changes, axonal growth, synaptic plasticity and neural function in rodent models of neurotrauma. This experimental approach builds on my previous work in the field.

Medium-term (within 5 years)

ii) Modulation of key molecular pathways to enhance neurological recovery in these animal models. In the medium-term, we will test potential strategies that may enhance repair, regeneration and functional recovery in pre-clinical models of nerve and spinal injuries. This benefit will specifically be achieved using introduction of pharmacological agents, gene therapy, biomaterials, stimulation of neural circuits and/or manipulations of sensory factors, such as enriched environments, that can modulate neural plasticity.
Long-term (over 5 years)

Development of novel pharmacological or gene therapies for treating these diseases in the clinic.

Progress to the clinic for gene therapy or novel pharmacological treatments for these neurological diseases is possible in five years and will be conditional to the successful demonstration that key novel molecular pathways can be modulated with novel drugs or viral vectors in animal models being safe and effective. Only after this essential groundwork in animals, will we be able to transfer this knowledge in human clinical trials.

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

We work closely with clinicians and those conducting human experimental medicine trials to try and facilitate rapid translation of major findings from bench to clinic. For basic work, we will share all data generated from this project freely with both long-term collaborators and the wider scientific community as in previous work. We will use pre-print online repositories to publish our work as soon as possible before peer-review. We are also committed to the publication of solid science leading to unsuccessful approaches since these can be very important to steer research in the right direction or away from possible failure.

Species and numbers of animals expected to be used

- Mice: 5000
- 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.

Rodents are among the simplest mammals to study functions that depend on the sensory and motor skills and on the integration of the cortex and spinal cord function. In vivo studies in rodents are needed in a highly wired and regulated complex environment such as the nervous system to obtain valuable mechanistic insight for human neurobiology. The use of rodents is further justified as many neural-circuit features relating to investigated areas of research (injury/pathology/plasticity and repair) are comparable between rodents and humans, making the investigation of preclinical models directly relevant for developing understanding, related to human disease progression. The rodent brain and spinal cord are also very similar to the human nervous system, displaying comparable impairments after spinal injury thus representing a highly suitable species to investigate mechanisms and regenerative treatments. Additionally, metabolic and gene regulation is much dependent upon the cell and injury signalling context; cell lines cannot be a valid alternative here. Our proposed approaches are preclinical models of disease progression that can be reliably tracked at the cellular and sub- cellular levels over time in vivo, which is not possible in either reduced preparations or human experiments. Therefore, the use of laboratory animals in this instance is unavoidable.

Mice are the species of choice in biomedical research because production of transgenic animals is already well refined. We use mice in our research when asking research questions that target the cellular and molecular mechanisms of injury and regeneration, because transgenic mice offer the possibility modify the biological system in a controlled manner and study the response. The research community has developed numerous genetically stable lines of transgenic mice, which makes the use of mice essential for this proposal. On the other hand, we also plan to use rats for their higher level of similarity to humans in terms of spinal cord injury pathology. Transgenic rats are scarce, so they are not optimal for mechanistic research, but their cellular and molecular response to spinal cord and nerve injuries is more similar to humans than that of mice. Therefore, it is more relevant to use rats when investigating treatment strategies with the potential of being translated to humans applications.

Additionally, our work focuses on animals across the lifespan given that nerve and spinal

injuries occur in young as well as aged individuals and their repair ability differs across the lifespan, decreasing with aging. Therefore, we need to contribute to further understanding the pathology of nervous tissue injury across the lifespan, as well as finding treatment strategies to improve regeneration in all age groups.

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

We use adult (2 - 15m) and aged (15-24m) wild-type mice and rats as well as genetically modified mice that capture key features of the age-dependent ability for repair after nerve and spinal cord injuries that is conserved between animals and humans.

In a classical scenario, animals may be deeply anesthetised, undergo a nerve or spinal cord injury, then once awake, they will receive treatment drugs via intraperitoneal route weekly for 4 weeks, undergo two behavioural tests and be killed to dissect the tissues of interest using a humane approved method. The total duration of this experiment can be of up to 8 weeks, including the behavioural experiments.

In the worst-case scenario, 20% of animals will undergo aging up to 20 months, diet change, irradiation and intravenous cell transplantation, sciatic nerve injury, spinal cord injury, neuronal tracingin the spinal cord, brain or nerve (to fluorescently label the injured neuronal tracts), treatment drug administration by oral gavage or intraperitoneally for 4 weeks, three behavioural tests and then be killed to dissect the tissues of interest using a humane approved method (this sequence, in the worst case scenario, includes up to 4 surgical events). The total duration of this experiment can be of up to 22 months, including the behavioural experiments.

Alternatively, after a nerve or spinal injury, animals may undergo one of a series of neuronal imaging techniques that measure in vivo neuronal activity and neuronal growth in the intact spinal cord. In this work, when imaging the spinal neurons directly, a spinal window will be implanted under general anaesthesia, during this step we may also inject a fluorescent reporter of neuronal structure/activity, electrodes (either on the skull or in the brain) or a small light-weight spinal mounting for later microscopy. In case of spinal window implantation, post-surgical imaging procedures could occur in the first days after injury or weekly for a duration of up to 8 weeks. The duration of the experiment could sum up to a total of 10 weeks including surgical procedures and stabilization of the implant.

Animals are allowed to recover for a variable span of time after nervous system injury between one to several weeks and given post-surgical analgesia. After recovery animals may undergo periods of sensory environmental enrichment. Enrichment involves stimulating with sensory inputs in the home cage by adding toys or objects for the mice to play with. We do not anticipate any adverse effects due to environmental enrichment.

Animals may also undergo a period of learning prior to other procedures using standard behavioural approaches including sensorimotor tests such as gridwalk, rotarod and locomotion in an open field. The goal of this pre-surgical training is to get the animals acquainted with the behavioural test procedures while in pre-injury, healthy conditions.

Nerve or spinal injury, imaging, sensory manipulations and behavioural assessments can be accompanied by delivery of certain drugs that are thought to act as interventions in the disease process. Additionally, electrical brain stimulation as a plasticity inducing treatment, or electrophysiological activity recording using electrodes may be used to evaluate the degree of recovery from injury in terms of neuronal signal conduction. These experiments

would be done through an implanted electrode or through temporary electrodes, respectively. Electrophysiological recordings are typically performed in terminal conditions, so before the animal will be humanely killed for tissue dissection.

Some of our experiments are longitudinal and so may span several months to capture the ageing or disease process. However, the number of procedures is closely regulated so that a typical animal will undergo no more than: one mandatory surgery and possibly one optional surgery, one dietary regimen, one therapeutic administration in a variety of frequencies and duration depending on the drug, limited behavioural assessments and limited periods of imaging, depending on the imaging method.

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

Old age can be associated with increased incidence of cataracts, arthritis, obesity, spontaneous tumours, ulcerative dermatitis. Based on our previous experience, we anticipate that no more than 15% of mice in this protocol will exhibit such clinical signs of moderate severity. If any symptoms of ill health appear after performing procedures, the animal will be monitored more closely and specific interventions to address each symptom will be performed following veterinary advice. If no improvement is seen within 2 days, animals will be killed to dissect the tissues of interest using an approved humane method. Aged animals may die unexpectedly without showing prior signs of ill health, we estimate based on prior colony numbers that this may occur in between 1 – 2 % of aged animals.

Surgery such as nerve or incomplete spinal cord injury will lead to moderate severity. This includes pain, which will be controlled with analgesia, and incomplete paralysis for several weeks, however the animals will be able to drink and feed independently. The type of paralysis that animals may experience after a sciatic nerve crush or moderate spinal cord injury is lack of motor control of the hindlimbs (unilateral or bilateral, depending on the location of the injury) and loss of sensory perception in the same area. The loss of function will be temporary (1-2 weeks) in case of crush injuries, but in case of sectioning of one side of the spinal cord on unilateral nerve sectioning, paralysis in one hindlimb would be chronic, yet not affecting its autonomy. They might have transient weight loss and reduced general activity for the first 2 to 3 days after surgery after which these will normalise. A maximum of 10% of animals undergoing surgery will receive a complete spinal cord injury (medial deep and long crush or transection) leading to permanent paralysis after surgery until the end of the protocol (up to 22 weeks after injury in the longest case involving the development of a chronic injury + treatment). This will be necessary to model more severe injuries observed in humans. These animals will also have bladder paralysis so they will be monitored and their bladder emptied twice daily for the first week and once daily thereafter. The severe qualification for animals undergoing this section of the protocol is due to the lack of spontaneous functional recovery after receiving this type of injury, which results in chronic loss of motor and sensory function below the level of injury. Despite the fact that animals will be given multiple types of pain relief after surgery, severe spinal cord lesions are not associated with enhanced pain compared to incomplete lesions, due to the loss of sensory input, nor with sudden deaths.

Optical injury will include an optic nerve crush, which will lead to impaired vision or progressive vision loss. Additionally, animals may undergo a lens injury to stimulate regeneration. The lens and optic nerve injuries could cause a moderate cytotoxic immune response, which may lead to swelling and higher temperatures around the lesion site. Modest swelling around the lesion site will be treated by cleaning and aseptically prepare

the area and by giving local anesthetic and antibiotics when needed and under NVS advice. With the optic nerve injury (only on one side), animals will become blind in one eye. This however does not impair their activity and independence as well as access to food and water. This injury model based upon our direct experience and that reported in the literature is very well tolerated. Other possible, but rare adverse events are reduction in appetite, ruffled fur, ocular or nasal discharges, behavioural abnormalities. Although a very rare occurrence, animals might develop an enlarged globe after ocular surgery. Post-surgical infections are rare and should be less than 1%. If any symptoms of ill health appear after performing procedures, the animal will be monitored more closely and specific interventions to address each symptom will be performed following veterinary advice. The animals will be humanely killed within one day if no improvement is seen.

Drug or gene delivery, neuronal tracing, imaging and behavioural testing are highly refined procedures in my group that are not expected to result in additional adverse effects. But drug administration and neuronal tracing may still lead to bleeding or subdued behaviour with decreased responsiveness.

Based on our previous experience, we anticipate that no more than 15% of mice in this protocol will exhibit such clinical signs of moderate severity. If any symptoms of ill health appear after performing procedures, the animal will be monitored more closely and specific interventions to address eachsymptom will be performed following veterinary advice. The animals will be humanely killed within one day if no improvement is seen.

Administration of agents affecting nervous system functions and/or potential control substances are mild and will have minimal adverse effects, however animals will be closely monitored during the period of substance delivery. Insertion of a minipump may be associated with wound breakdown and infection. Other potential adverse effects may include hunched posture, abnormally increased or reduced activity, peritonitis or local irritation around the route of administration. If any of these symptoms appear after performing procedures, the animals will be monitored more closely and specific interventions to address each symptom will be performed following veterinary advice and if they do not respond to treatment/supportive measures within 1-2 days, will be humanely killed. Maximum treatment frequency and duration will be daily for a period of three months after beginning of treatment.

Animals given substances to induce transgene expression (e.g., tamoxifen or doxycycline) will be carefully monitored, but no adverse effects are anticipated at the doses to be administered. In rare cases adverse effects may occur due to the method of dosing (including administration of the vehicle substance) resulting in local inflammation around injection sites, peritonitis, aspiration pneumonia or reduced food or water consumption leading to weight loss or dehydration. Other effects include gastric ulceration, abdominal hernia, alopecia and enlarged testes (so far, these being clinical signs associated with tamoxifen administration). Incidence of adverse effects is usually rare. Mortality rates are expected to be below 1%. To screen for adverse effects animals will be checked and monitored, at least daily and weighed regularly. Animals displaying signs of peritonitis (hunched posture, reduced activity) or aspiration pneumonia (respiratory distress) will be immediately humanely killed. Other signs of pain and distress include separation from cage mates, facial grimace, ocular or nasal discharge, piloerection and diarrhea, mice will be monitored and humanely killed (through an approved killing procedure listed under Schedule 1) if these do not resolve within one working day.

Spinal cord window implantation under general anaesthesia will be accompanied by

appropriate level of anti-inflammatory/multi-modal peri-operative analgesia. Following recovery from anaesthesia mice will be returned to their home cage. Analgesia will be given as required after surgery. After surgery, animals will be kept in a warm environment and monitored until the experimenter is confident that the animals do not suffer any adverse effects of the surgery, i.e. until ambulant and eating or drinking.

During the first few days of the recovery process animals may be provided with soft food to facilitate feeding in case of difficulties reaching the regular food.
Repeated imaging sessions in awake animals will typically last 90 minutes, and very rarely more than two hours. For anaesthetised animals imaging/electrophysiological recording sessions will rarely exceed two hours, in the instances when they do (<5%) we will continue to closely monitor anaesthetic levels and physiological parameters such as temperature and heart rate. In instances where the animals become unstable the experiment will be terminated and the animal humanely killed, we anticipate that this will be extremely rare in our experimental programme (<1 %). For high frequency anaesthesia (more than once in a 24- hour span), inhalation anaesthetics will be used.

Food restriction can be required to maintain motivation to perform some automated behaviour tests and should not normally lead to adverse effects. In these instances, restricted access to food will be performed for only 1-3h before the test, not chronically throughout the duration of the experiment.

Therefore, it is highly unlikely that the weight of the animals will be affected. However, animals will be closely monitored and will be weighed every 2-4 days during experiments. If at any time, any animal drops up to 90% of its free-feeding body weight, it will immediately be given food ad libitum before the tests and monitored daily until its weight is above 90%. The animals will be humanely killed if no improvement is seen or if weight loss reaches 15% of the original body weight.

Animals can undergo irradiation, to deplete them from bone marrow hematopoietic cells, followed by cell replacement therapy to prevent any effects of reduced haematopoiesis. Mice that receive high doses of irradiation in this protocol receive a sufficient number of haematopoietic cells to allow for complete engraftment and are not expected to show major symptoms. However, a subset of animals undergoing irradiation may experience temporary weight loss, diarrhoea (due to damage of the intestinal epithelium) and general signs of ill health (piloerection, reduced movement and reduced appetite). Weight loss (usually 5-10%) typically peaks at day 5-10 post irradiation. Damage to the haematopoietic system may cause bone marrow depression in low doses and its destruction in high doses can result in increased susceptibility to infections, leucopoenia, platelet deficiency resulting in haemorrhages (immediately) and anaemia (pallor). Irradiation commonly affects pigment production leading to dark furred mice producing grey/white coats, this does not affect the animals' welfare. Aged animals may experience the same adverse effects, but exhacerbated (i.e. with earlier onset, longer duration or increased intensity).

The effect of the circadian cycle on neurological regenerative ability will also be studied because we previously observed that the ability of neurons to regrow varies along the circadian cycle. These experiments have been designed to maintain light and temperature conditions that avoid the disruption of the rodent circadian cycle. Experimental procedures described in other steps of this licence could be performed on these animals at any time point within the 24 hours daily cycle. No adverse effects are expected from the circadian control 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)?

Approximately 50% of the mice numbers are in our breeding protocol: mild severity (in practice, the severity these animals will undergo is subthreshold).
Approximately 45% of the mice used for experiments will undergo surgery of moderate severity, the remaining 5% will be severe.

Of the rats used in this proposal, 90% of them will be undergo procedures of moderate severity and 10% of them will undergo procedures of severe level severity. Moderate severity in this context means that the mice/rats will be quiet and move less for up to five days after surgery. Some will have partial limb paralysis until the end of the protocol up to eight weeks after surgery, but this will not impair their daily activities, drinking or feeding. The severe protocol means that mice/rats will have permanent paralysis after surgery until the end of the protocol. The mice/rats will be given multiple types of pain relief after surgery. Mice/rats might lose a bit of weight but will typically regain that weight within two to three days.

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

- Killed

A retrospective assessment of these predicted harms will be due by 13 January 2029

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?

Investigating the repair ability following nerve and spinal cord injury and identifying new molecular targets for clinical treatment is the mission of this project license. In order to gain any biological data of relevance to these human disorders we need to use animal models of nerve and spinal cord injury that are a reliable and comparable neuroanatomical species to humans. Additionally, these injury models in animals are a faithful representation of the anatomical, molecular, cellular and behavioural consequences that are observed in humans, including by showing long-term neurological impairment. Animals also represent one of the only feasible strategies to study the complex processes that follow nervous system injury, as this is not well captured by in vitro assays, computational modelling, stem cell related work alone or even post-mortem human brain experiments.

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

I am taking every possible step to embrace feasible replacement options. This includes the use of hiPSC models including organoids for studying aspects of human disease, in silico modelling of neuronal outgrowth dynamics to model regeneration and manipulations of brain activity with non- invasive stimulation technology in humans that may enhance plasticity of spared neurons after spinal cord injury.

Why were they not suitable?

The proposed future work uses experiments involving nerve and spinal cord injuries across the lifespan, which is not feasible in preparations without vasculature such as cell cultures including 3D organoids, reduced preparations or in silico modelling given the complex multifactorial nature of the nervous system and of post-injury related processes. In addition, mice can be used in conjunction with other technologies for reporting or manipulating neuronal growth and activity that impacts behavioural phenotypes such as sensory and motor performance. Currently, animal experimentation is the only approach available to detect molecular, cellular and systemic (in the blood or distant organs) responses after nervous system injuries and to detect the impact of interventions on anatomical repair and behavioural phenotypes. These preclinical models of disease progression or recovery can also be reliably tracked at the cellular and sub-cellular level over months in vivo, which is not possible in either reduced preparations or human experiments.

A retrospective assessment of replacement will be due by 13 January 2029

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 animals to be used in each protocol based upon our previous research in similar experimental settings. Additionally, these estimates are based on previous work under the last project license using similar workflows and staffing levels. We estimate that objective 1 will require ~1750 mice across 5 years: this objective aims to determine the molecular mechanisms responsible for neural response to spinal and nerve injury, including for axonal regeneration, in specific neuronal populations. Objective 2 aims to provide molecular and cellular based causality of the identified pathways with cellular and biochemical studies and will require ~1250 mice across 5 years for culture preparations. Objective 3, which aims to describe the role of regenerative pathways identified in objectives 1 and 2 on functional recovery, will require ~650 mice across 5 years to perform behavioural studies after manipulation of pathways relevant to repair for nerve and spinal cord injury.

Lastly, objective 4, which aims to investigate the regenerative potential of said pathways in the context of ageing, will require ~350 mice across 5 years. Objective 5 aims to investigate regenerative pathways associated with different nutritional regimens and will require ~750 mice to perform experiments similar to those in objectives 1 and 2. Finally, objective 6 aims to study the role of the circadian clock upon on the regenerative capacity and gene expression, and will require ~250 mice. A much smaller number of rats, ~520 across 5 years will be used to fulfil the above objectives only in a subset of experimental settings to provide validation of the most promising work done in mice in another clinically relevant animal model of nerve and spinal cord injury.

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

Whenever experiments have to be undertaken with a new experimental design, we will carry out pilot experiments with low animal number (such as n=4) to gain preliminary biological insight or establish a technique at specific end points. Pilot studies provide essential estimates of variability of observations, a critical factor in statistical power calculations used to predict group (sample) size. From previous experience we know that group sizes in the types of experiments we will undertake can typically range anywhere from n = 6 – 12 animals depending upon the level of behavioural improvement and variability observed. We will use statistical power calculations based on our pilot data and as reported in the literature for each model used as a guide to whether our hypotheses can be proven or not with a reasonable number of animals (groups of n = 15 will be our upper limit i.e. specific spinal cord injury lesions where complex sensorimotor behavioural effect is being measured). For specific calculations we will use online free experimental design assistant tool (EDA) available from NC3Rs. This includes the possibility to organise experimental planning, blinding, and randomisation and derive animal numbers based upon the power and statistical test of choice. We have been using this tool for the past 5 years during our current license and it has allowed us to reduce the n of animals in several instances (where we had previously based our assumptions on published work only), while making the experimental design stronger and data more reproducible. While adhering to the ARRIVE guidelines, we will also use a common-sense approach will also be adopted whereby if our treatment strategies were clearly having no effect those particular experiments would cease forthwith and our strategy revised or revoked. Likewise, should our treatment strategies be obviously therapeutic the smallest statistically valid group sizes would be reported. While we have designed all experiments to optimise power and minimise usage by considering the expected effect size whilst maintaining statistical power, the advice of statisticians will be sought when necessary. In addition to careful calculation of predicted animal usage, additional experimental and technical approaches will be employed to meet the needs of reduction. For example, imaging approaches will be used to monitor axon growth in vivo (rather than tissue sections) so that data from more cells/axons per animal can be collected thus reducing animal numbers.

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

The following measures have been and will be undertaken to optimise the number of animals:

Maximise the efficiency of breeding by following published guidelines (https://www.ncbi.nlm.nih.gov/books/NBK500423/; https://www.jax.org/jax-mice-and-services/customer- support/technical-support/breeding-and-husbandry-support/general-

husbandry-tips) and guidance from the Home Office.

Pilot studies will be undertaken whenever effect size needs to be established because not present in the literature or available from previous similar experimentation

In silico computer modelling and machine learning algorithms will be used to model the effect size of regenerative interventions whenever possible

We will use several in silico analysis (Pathway analysis, Protein interaction analysis software) that contribute to reduce the number of animals by focusing the research questions

Where appropriate, data will be made freely available to other researchers and tissue will be shared through collaborative work, thus supporting reduction to minimise unnecessary use of animals.

A retrospective assessment of reduction will be due by 13 January 2029

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 experiments will include moderate protocols in 90% of animals undergoing experimentation.

In fact, we strived to choose the most representative animal models that resemble these human diseases and are good predictors of effectiveness of treatment under investigation by using moderate severity protocols and resorting to substantial severity in 10% of animals only, when a clinically promising treatment needs to be tested in a complete spinal cord lesion that resembles complete spinal cord injury in humans with permanent disability.

In order to minimise animal sufferings in our injury models we will limit the extent of the lesion to what is strictly needed to study neuroregeneration pathways that may be useful for humans.

Specifically, (1) we will always aim to lesion selected spinal cord segments versus complete lesions; (2) we will perform a milder optic nerve crush lesion and not a complete transection; (3) we will perform sciatic nerve crush whenever possible instead of

transection, although the transection of peripheral nerves does not incur into a severe protocol either.

Whenever needed, we will use imaging as non-invasive procedure to assess function and the terminal electrophysiology will be conducted under terminal non-recovery anaesthesia.

Additionally, suffering will be principally minimised by optimal operating technique and providing adequate analgesia and good post-operative care. Whenever there will be a choice of route of administration of drugs or virus the least invasive will be adopted. No intra-muscular route of administration will be adopted unless obsoletely necessary.

Pain and distress will be carefully monitored after lesion and appropriate measures will be taken if signs of pain/distress are observed.

Based upon the experience gained in our current license:

We used developed, including after discussion with our veterinarians, an optimised analgesic protocol:

Peri-operative administration of standard analgesics (e.g., buprenorphine) Peri-operative administration of carprofen or analogue compounds for 2 days, and if signs of pain are noted, this can be extended until these resolve.

Intra-operative administration of bupivacaine (Marcain) or analogue compounds for local anaesthesia. This will last approx. 4-8 hours

This can be combined with lidocaine, which is quicker acting, but wears off after approx. 20mins to have the full effect under anaesthesia.

This mix can be applied after the initial skin incision to remove the pain of this for the first few hours post-operatively and can also reduce the amount of anaesthetic needed for surgery thus refining the procedure.

We have refined our skin preparation protocol, using more efficient hair trimmers and surgical wound closure is regularly reviewed with NVS/ designated trainers to ensure the most refined method is used. Altogether these refinements result in better welfare of the mice.

The most common adverse effects from the surgery would be infection that is expected to occur in a low percentage of animals (approx. 1%). The animals will be closely monitored throughout and treated humanely in consultation with veterinary staff in the event of pain or infection.

When irradiation is needed to deplete the hosts haematopoietic cell pool, the irradiation doses to be used will be the lowest effective dose to meet the scientific objectives. The protocol will also be immediately followed by cell transfer to replenish the cell pool and prevent consequences associated to immune depletion.

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

Mice are the most commonly used species for the type of procedures detailed within this application. Both mice and rats have been, and continue to be, used extensively in

neurodegenerative and neurotrauma research, including in neuroregeneration research for models of nervous system injury. Nerve and spinal cord injury animal models will be used, the PI has worked and published in these injury models for over 20 years. They allow reliable data for comparison in humans in terms of basic neurobiological mechanisms, neurophysiological correlates and behavioural testing. These rodents are relatively low order sentient animals (i.e., compared with non-human primates, cats, dogs, etc.,) however these species are accepted by the scientific community as useful animal models for both the basic and translational research we intend to carry out. In addition, based upon the need for data cross comparison and the availability of a large number of transgenic lines for mice, mice are our species of choice. Rats will be used only in the final step of the pathway to test potential therapeutic interventions in contusion spinal injuries or nerve injuries, once robust evidence has been generated using in vitro models and in vivo mouse models. This final step will serve to generate a clinically relevant peripheral or central neuronal regeneration evidence evidence base prior to clinical trials/translational studies.

Mice, due to their small size leading to marked differences in bioavailability, are not appropriate for this final step of the testing pathway for axonal injuries; rats have been shown to be an important intermediary to clinical translation and could help protect patients from treatments with minimal efficacy and potential adverse effects of these treatments.

Our experiments investigate the molecular and cellular mechanisms that underpin repair after nerve and spinal cord injuries occurring in clinical settings in young adult until aged individuals. Animals are required to be sufficiently sentient to perform behavioural tasks and respond/adapt to injury and to pharmacological or genetic manipulations. In addition, we are interested in developing a better understanding of age-related regenerative decline therefore work needs to be completed in adult - aged animals as well as relevant preclinical rodent models of nerve and spinal cord injury across the lifespan.

The use of less sentient animal models, such as zebrafish or flies, is not feasible for the reasons stated above. The molecular and anatomical characteristics of these animal models are too different from humans and other mammals, as they retain their regenerative capacity and their response to neurotrauma is cellularly and molecularly different. Therefore, they could be useful for basic and evolutionary biology questions, but they are not appropriate for translational and therapeutics discovery research.

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

Rodents are the species of choice in biomedical research because production of transgenic animals, relating to rederivation, breeding and colony management is already well refined. All experimental procedures meet the requirements of the currently held PPL, specifically focusing on cumulative severity, experimentally defined end points and limiting the number of repeated interventions. Many of the proposed future work plans aim to refine experimental approaches:

the use of environmental enrichment that has been shown to enhance the general animal welfare

training animals in sensory and motor tasks to maximise their welfare and recovery after injury

we intend to use state-of-the-art engineering and 3D printing facilities to support

development of the lightest possible head and spinal cord mounting devices.

We aim to modulate the level and frequency (to daily as needed) of post-operative monitoring depending on the severity of the spinal injury and related disability

Peri-operative analgesia, which is already implemented in the current protocol, will be included and kept up to date with the advice of the NVS to minimize pain and suffering.

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

We will follow LASA and NC3Rs guidance as well as regularly consulting our local AWERB.

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

I work closely with the NVS and veterinary support team, I also have close contacts with my local AWERB and receive regular updates on 3Rs conferences and opportunities.

A retrospective assessment of refinement will be due by 13 January 2029

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. Non-rodent/large animal models of surgery, toxicity and safety

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

Safety, Efficacy, Surgical Models, Livestock species, Dog

Animal types	Life stages
Cattle	juvenile, adult, embryo, neonate
Pigs	juvenile, adult
Sheep	juvenile, adult, neonate, embryo
Goats	juvenile, adult, embryo, neonate
Horses	juvenile, adult
Cats	juvenile, adult
Beagles	juvenile, adult
Chickens	juvenile, adult
Rabbits	juvenile, adult
Minipigs	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?

This project uses domestic animal species, which for the purpose of this project are defined as those species generally regarded as farm livestock, i.e. cattle, pigs and minipigs, sheep, goats and poultry (chickens, turkeys); or as companion animals, i.e. dogs; or species which may fall into either category,
i.e. rabbits.

This project aims to determine scientific and/or regulatory endpoints in the assessment of efficacy, safety and toxicity of human and veterinary pharmaceuticals, and medical devices, for regulatory submission/to satisfy governmental regulatory requirements, for safety assessment and in the case of veterinary or human pharmaceuticals only, for candidate selection.

These studies are run to satisfy the requirements of UK/EU (and sometimes international regulatory authorities) who are independent of governments) which require the testing of pharmaceuticals in a non-rodent species. Study designs are based on OECD, ISO and ICH guidelines for human Pharmaceutical and medical device testing, and VICH guidelines for veterinary pharmaceuticals.

No cosmetic products or chemicals that are exclusively intended to be used as ingredients in cosmetics will be tested.

A retrospective assessment of these aims will be due by 12 January 2029

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?

New medicines or medical devices have the potential to be of benefit in new or improved disease treatments in both humans and animals. Before potential new medicines/devices are administered to humans or animals their safety must be evaluated. This testing is a mandatory legal requirement and provides information on risks to people/animals taking new medicines. Often, the new pharmaceuticals we test in this programme will be designed to be better than existing treatments, possibly with fewer or less severe side effects.

At present there are no alternatives that don’t use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity or safety assessment in animals of human and veterinary pharmaceuticals, or medical devices. Species used on this project include domestic livestock such as pigs, goats, cattle and birds.

Dogs will be used only where the purpose of the study/programme of work cannot be achieved using any other species. In nearly every case, the justification for their use is that they are a target species for veterinary/animal health products, where evaluation in the

target species is mandatory.

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

The principal benefit of the project is the provision of data to facilitate sound decisions on safe/effective product development and appropriate regulatory decisions on clinical trial approval or marketing authorisation for new medicines and medical devices to which humans or domestic animals will be exposed, thus contributing to their protection and safety.

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

Human and animal patients will benefit from these studies as this work will contribute to the development of new drugs and devices that help alleviate human and animal conditions. These new drugs or devices 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 devices, and that knowledge may be used to develop further new drugs/devices.

One of the key benefits is the production of data that is required by regulatory authorities, to ensure medicines can be dosed safely, or devices applied safely to humans or animals. The drugs/devices that will be tested are for a variety of conditions, in some cases where there is an unmet clinical need to treat such conditions.

In addition, the models on this project may be used to assess the safety or other in life properties of a new drug,and find a dose that causes no adverse effect. This is important when planning future trials in humans or animals, to make sure any starting dose in a clinical trial is safe for the patients taking it.

Our customers will also benefit, as the data we generate will allow them to progress their new drugs into clinical trial, or otherwise if they are found to have adverse side effects.

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 regulatory purposes or to support regulatory purposes (e.g. to support candidate selection or to support drugs/devices 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 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 pharmaceuticals and devices that can be administered to humans and animals, (either by informing on safety and allowing to progress to clinical trials or preventing pharmaceuticals/devices reaching the market due to 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

- Rabbits: 200
- Cattle: 230
- Sheep: 570
- Pigs: 1020
- Minipigs: 1020
- Goats: 160
- Other birds: No answer provided
- Beagles: 480

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 uses domestic animal species, which for the purpose of this project are defined as those species generally regarded as farm livestock, i.e. cattle, pigs, sheep, goats and poultry (chickens, turkeys); or as companion animals, i.e. dogs; or species which may fall into either category, i.e. rabbits.

The evaluation of safety/efficacy of veterinary medicines and other animal health products, and safety of other substances to which they may be exposed, is self-evidently best achieved through testing in the target species. For studies in which non-rodent species are used as models for the assessment of human safety, species selection is made on a case-by-case basis according to various criteria including physiological, morphological and anatomical similarities with humans. In cases where conventional non-rodent models (pigs or, where justified, dogs or non-human primates) are unsuitable an alternative is needed, and large ruminants, particularly sheep, may often fulfil the necessary validity criteria for use as a toxicological model.

Pigs, sheep and goats are all well-established models for surgical studies of various types, again based on suitability/validity criteria (for example, sheep and goats are used extensively in orthopaedic research because of similarities in their bone architecture and bone regeneration processes to those of humans).

Dogs will be used only where the purpose of the study/programme of work cannot be achieved using any other species. In nearly every case, the justification for their use is that they are target species for veterinary/animal health products, where evaluation in the target species is mandatory.

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

In general, animals are dosed/treated by the intended/likely route of human (or target animal) exposure, and observed regularly to monitor appearance, behaviour and clinical health. The main study types that are performed under this licence for each class of test item are:

Veterinary medicines/animal health products:

Efficacy – animals are dosed at clinically relevant doses and observations on expected parameters of efficacy are made. In some cases, it may be necessary to administer a challenge treatment to elicit a condition against which efficacy can be assessed – for example an experimental infection with intestinal nematodes in sheep to test a veterinary worming medicine.

Target animal safety - Animals are dosed at clinical doses and low multiples thereof and observed regularly. Typical investigative procedures are similar to diagnostic procedures that might be used medically to monitor progress of a human patient (e.g. collection of blood samples for laboratory investigations, or ECG monitoring to assess heart rate/function). Terminal investigations will involve sampling and processing tissues for pathological assessment.

Pharmacokinetic, metabolism and residue studies – Animals are dosed at clinically relevant doses and observed regularly. The metabolism, fate and distribution of the test item is investigated by analysing samples of blood, excreta, expired air, milk, eggs and tissues taken post mortem, as appropriate.

Human pharmaceuticals:

Safety/Toxicity studies – Dose levels for definitive toxicity studies in animal models are determined in preliminary studies and are selected to investigate mechanisms of toxicity and a safe exposure level (no-effect level) that can be related to expected clinical exposure. Typical investigative procedures are similar to diagnostic procedures that might be used medically to monitor progress of a human patient (e.g. collection of blood samples for laboratory investigations, or ECG monitoring to assess heart rate/function). Terminal investigations will involve sampling and processing tissues for pathological assessment.

Pharmacokinetic, metabolism and biodistribution studies – Animals are dosed at clinically relevant doses and observed regularly. The metabolism, fate and distribution of the test item is investigated by analysing samples of blood, excreta, expired air and tissues taken post-mortem, as appropriate.

Wound healing models:

Surgical wound healing models are included in this licence and are used to evaluate the effects of treatments/dressings/devices on the rate and quality of healing. The surgical procedures are performed under general anaesthesia with full monitoring of vital signs and pre-/post-operative preventive analgesia and antibiotic treatment. Animals are monitored closely during surgical recovery and appropriate investigations are carried out similar to those used in safety/toxicity studies. Terminal investigations will involve assessment of healing at the surgical sites and sampling/processing of tissues for pathological assessment.

The protocols for all the above studies have a moderate severity classification. However, most animals are expected to experience no adverse effects, or only mild effects such as slight weight loss or transient discomfort due to dose injection or blood sampling. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss/reduced activity. A very small number of animals may experience severe adverse effects without intervention, but humane end-points are

applied to avoid this and to prevent unnecessary suffering.

Some animals may be re-used or rehomed. Where re-use of animals is considered as a strategy to reduce the numbers of animals used, this is assessed against the potential overall welfare harms to the animals, taking into account their overall lifetime experience. After each use, animals are assessed for suitability for keeping for re-use, and any animals showing significant adverse effects will not be re- used.

The rehoming of animals as companion animals is subject to careful assessment and confirmation of health, suitability for rehoming including appropriate socialization procedures, and confirmation that the animals do not pose any risk to human health, animal health or the environment. In addition, for animals released back to commercial livestock use, checks are made to ensure that any other applicable legislative requirements (e.g. DEFRA requirements on use of animal health products and withdrawal periods for food producing animals) are met.

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

Animals are dosed by the intended/likely route of human or animal exposure (for example oral administration, injection, infusion, and observed regularly to monitor appearance, behaviour and clinical health.

When dosing an animal by injection or taking blood, the degree of pain or discomfort an animal feels is similar to what a patient would feel having an injection administered by a doctor.

Dosing with drugs may cause adverse effects in some studies. Experience from the last licence shows that the majority (~90%) of animals display only mild severity with the remaining 10% displaying moderate severity.

Lethality and/or severe effects are not expected to occur, in any of the protocols in this licence.

We observe our animals at least twice a day, and the people who do this know the signs when an animal is unwell. If an animal is unwell, we would check it more frequently, and consult vets and other senior animal care staff for advice and guidance in its care.

Most animals are expected to experience no, or only mild, adverse effects such as slight weight loss during the course of the study. A small percentage of animals may show more significant adverse effects, such as more marked weight loss, reduced activity, vomiting or tremors. No animals would be expected to die or to suffer prolonged adverse effects as a result of the procedures, and where necessary early humane endpoints are applied, under veterinary guidance as necessary, to prevent this; such endpoints might include interventions to discontinue dosing, or to provide supportive treatments, or if necessary to humanely kill the animal.

Animals in surgical wound healing studies are normally regarded as experiencing moderate adverse effects (though they are given appropriate pain relief medication) and may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients. However, supportive treatments are given to eliminate or minimise these adverse effects, and humane endpoints are again

applied. All surgical procedures are performed under anaesthesia, with full peri- and post-operative analgesic cover to reduce/eliminate as far as possible any pain or discomfort during surgical recovery, as would be the case for a human patient.

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, about 50% of animals were classified as having experienced mild severity, around 35 to 40% were classified as moderate. The moderate severities in the last project would have been due to treatment-related signs of moderate severity (mostly in prelims) or because a surgical procedure was involved. It is impossible to predict the proportion of severities expected on a service licence like this, as this will be dependent on what study types we are asked to perform. However, a distribution between 'mild' and 'moderate' severities similar to those in the last project are anticipated.

All protocols on this licence are classified Mild or Moderate only, there is no intention to perform any procedures that are Severe in nature. Under the last project licence (at the time of writing) no animal had been classified as having experienced severe severity.

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

- Killed
- Kept alive
- Rehomed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 12 January 2029

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?

Human and animal pharmaceutical and medical device testing in animals is a mandatory legal and regulatory requirement and provides information on risks to people and animals taking new medicines and using such devices. At present there are no alternatives that don't use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment in animals.

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.

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?

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.

However, no studies in animals are conducted under this licence until an assessment has been made to determine that the specific study is necessary and justified, i.e. the study aims and objectives are consistent with the scope and purpose of the licence and cannot be achieved by any other means not involving the use of animals. This assessment will involve consideration of any potential non-animal alternatives, review of existing data on the test item and reference to any other relevant information (including literature review, in-house data, information on similar items).

Why were they not suitable?

Although there are in vitro tests that can model some parts of how drugs get into our bodies, and how our body deals with them, and can identify undesirable effects, for example, there is no series of in vitro tests that brings all these complex events together, as in the whole (animal or human) organism.

That is why we need to test new drugs in animals, as they have similar physiology and metabolic processes as humans, and that testing gives us a good idea what may happen if they are subsequently used in humans. For animal pharmaceuticals, the use of the target species is mandatory.

A retrospective assessment of replacement will be due by 12 January 2029

The PPL holder will be required to disclose:

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

Reduction

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

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

The numbers we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received from customers in the past. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

The numbers of animals used in each study are in some cases specified in the regulatory guidelines; where not specified, numbers are based on established minimum regulatory expectation, or on scientific estimates of the minimum numbers required to meet study objectives.

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.

For regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international guidelines where these exist. Otherwise, reference is made to standard study designs with input from the Department of Statistics, where appropriate, to identify the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. These internal designs are reviewed and updated in line with changing external guidelines and internal refinements that either minimise numbers or reduce severity.

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.

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, we use smaller groups of animals where applicable to get an idea of the doses (of pharmaceuticals) we need to use for the main studies. These preliminary studies are important as they give us confidence that the doses we are using are correct prior to testing them in larger groups of animals as required by global regulators. Similarly, pilot studies/proof of concept studies are often used when testing medical devices to assist in optimising the procedure in the subsequent definitive regulatory studies.

A retrospective assessment of reduction will be due by 12 January 2029

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 uses cattle, pigs, sheep, goats and poultry (chickens, turkeys) dogs and rabbits. Dogs will only be used when no other species is deemed suitable to meet the scientific and regulatory aims of a study.
Sequential testing, with review of findings at each stage and modification of subsequent stages as necessary, maximises opportunities for refinement to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals.

Where appropriate, positive reinforcement training (treat rewards) is used to encourage co-operation in (and minimise any stress of) handling/procedures. Environmental enrichments appropriate to the species are used within the animal facilities.

Animals are monitored for clinical signs of toxicity or other effects on their health and wellbeing, and in order to prevent unnecessary suffering, humane endpoints are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).

The models we use are the least invasive procedures, for the least amount of time necessary to get the information we need. They are carried out using standard and recognised techniques by fully trained staff. We also have veterinary clinicians on hand for advice and on the occasions we have to anaesthetise the animals and for general advice on animal welfare.

If we have to repeatedly inject animals or 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 place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. If we can take blood samples when an animal is anaesthetised, then we do so.

Surgical procedures will be carried out aseptically and to at least the Home Office minimum standards for aseptic surgery, and in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) (LASA is the Laboratory Animal Science Association).

All surgical procedures are performed under anaesthesia, with full peri- and post-operative analgesic cover to reduce/eliminate as far as possible any pain or discomfort during surgical recovery, as would be the case for a human patient. Supportive treatments are

given to eliminate or minimise any other incidental adverse effects of the surgical procedure (for example, temporary impaired kidney function following renal surgery) and humane endpoints are again applied.

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

There is a scientific and regulatory requirement for safety/toxicity/surgical data in non-rodent species, and in the target species for veterinary medicines (for example).
We use pigs in preference to dogs wherever possible; (a legal requirement in the UK), and dogs are only used where necessary to achieve the study objectives, i.e. when the dog is the target species, or the pig is unsuitable (for example due to species-specific differences from humans, confounding pharmacology or toxicological responses, or practical limitations due to anatomy or physiology).

Most of these studies require repeat dosing for days, weeks or months, to assess potential adverse effects in man or animals, so 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?

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet at least the standards set out in the Home Office Minimum Standards for Aseptic Surgery. Before we start surgery, we agree with a veterinary surgeon what pain killers or antibiotics the animals need both before and after the surgery. When animals are recovering from surgery, we give them extra heat and monitor them closely until they are fully recovered and showing normal behaviour.
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 urine collection), then we would habituate animals to this equipment prior to study use. Most animals habituate well to this equipment, but if they don't (rare) we remove them from the study.

If we have to repeatedly inject animals or 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 place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. If we can take blood samples when an animal is anaesthetised, then we do so. All personnel performing these procedures are trained to a high standard to minimise adverse effects.

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.

Refinements to improve the animals' experience include but are not limited to group housing, environmental enrichment, including novel toys and food treats, human interaction, acclimatisation and training to procedures, and calming measures such as stroking/gentle talking are used to help animals have a better experience of restraint. We have dedicated working groups on animal welfare for each species with a permanent brief to identify potential measures to improve animal welfare, and to trial such measures.

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

General:

Diehl et al. 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).

Pharmaceutical (human):

The conduct of Regulatory Toxicology and Safety Evaluation Studies. UK Home Office. 2005.

ICH M3(R2) (2009) Guidance on Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.

Olsen H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B and Heller A. 2000. Concordance of the Toxicity of Pharmaceuticals in Humans and Animals. Regulatory Toxicology and Pharmacology. 32 56-67.

Sparrow S, Robinson S, Bolam S, Bruce C, Danks A, Everett D, Fulcher S, Hill R, Palmer H, Scott E and Chapman K (2011) Opportunities to minimise animal use in pharmaceutical regulatory general toxicology: A cross-company review. Regulatory Toxicology and Pharmacology doi:10.1016/j.yrtph.2011.08.001

Surgical/Medical devices:

ISO 10993 Guidelines for the Biological Evaluation of Medical Devices: Part 1 (2009): Evaluation and testing within a risk management process; Part 2 (2006): Animal welfare requirements; Part 6 (2016); Tests for local effects after implantation; Part 11 (2017): Tests for systemic toxicity.

Veterinary/Animal health:

VICH Guidelines: GL43 (2008), Guideline on Target Animal Safety for Veterinary Pharmaceutical Products; GL46 (2011), Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Metabolism study to Determine the Quantity and Identify the Nature of Residues; GL48 (2011), Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods.

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.

A retrospective assessment of refinement will be due by 12 January 2029

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. Metabolic basis of microglia function in development

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

Neuroimmunology, Neuropsychiatric disorders, Maternal immune activation, Maternal infections, Maternal diet

Animal types	Life stages
Mice	neonate, pregnant, 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.

- 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 objective of this work is to determine how the metabolism of immune cells present in the brain of developing mice regulates their activity during normal development and after the activation of the immune system in their mothers (i.e., using mouse models of “maternal immune activation” or MIA). The ultimate goal of this project is to identify cellular and molecular pathways that can be modulated in immune cells to understand and treat major neuropsychiatric disorders.

A retrospective assessment of these aims will be due by 20 January 2029

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?

Evidence suggests that neurological and mental disorders (altogether “neuropsychiatric disorders”), such as autism spectrum disorders (ASDs), schizophrenia, and depression, are linked to maternal infection. Scientists analysed the medical records of ≈ 1.8 million pregnant women and their children and found that infection diagnosed in pregnancy increased the risk of children to develop ASD or depression in the following 41 years. Specifically, researchers found that there was a 79% increased risk of ASD among children and adults exposed to any maternal infection during pregnancy. This means that while 2/1,000 children developed ASD if there were no infections during pregnancy, this number increased to 5/1,000 if there was any maternal infection. Similarly, researchers found that there was a 24% increased risk of depression, which increased the number of depressed individuals from an average of 10/1,000 to 14/1,000 in case of any maternal infection. Several studies in animal models have confirmed that acute inflammatory episodes in pregnant females are sufficient to trigger brain alterations in the adult offspring, especially in those specific areas that are found to be affected in psychosis. From a mechanistic perspective, strong evidence supports the involvement of inflammatory molecules released by the pregnant female during infections (e.g., cytokines, such as interleukin 6 and leptin, an hormone with effects on inflammation), which can reach the foetal brain leading to long- lasting detrimental effects. These data strongly support the idea that maternal infections (resulting in the activation of the immune system in the mother) can lead to the development neuropsychiatric disorders in children and adults later in life.

Additional evidence also suggests that maternal diet during pregnancy may play a role in the development of neuropsychiatric disorders in children. Maternal diet can influence inflammation in the brain, which in turn causes adverse neurodevelopmental outcomes such as ASD. Obese/overweight pregnant women show indeed an increase in circulating inflammatory molecules. A recent meta- analysis confirmed a significant increased risk for ASD in children conferred by being overweight and/or obese during pregnancy. These data suggest that the high-fat diet (HFD), which is becoming widespread in most countries, could be one of the causes of the rising incidence of ASD in the general population.

Therefore, key areas for research are to understand the role of maternal infections and maternal diet in inducing neuropsychiatric disorders in the offspring.

Previous studies identified “dark microglia” (DM) as a specific immune cell type that is nearly absent from the brain of healthy young adult mice and is instead significantly increased in conditions of brain damage or inflammation. The number of DM sharply increases in mature mouse offspring after the activation of the immune system in their mothers (i.e., in mouse models of maternal immune activation or MIA). Further work also showed that DM are present in the human brain of patients with neuropsychiatric

disorders, where they contact and interact with the blood vessel and brain cells. Most importantly, recent data show that DM have a distinct way to produce energy (metabolism) and accumulate glycogen granules - a glucose storage - which is associated with a pro-inflammatory damaging activity of the cells of the immune system.

With this license, we wish to further explore how MIA caused by infection or diet regulates the interactions between the brain and the different types of immune cells (with a specific focus on DM). We will concentrate our efforts on studying the way DM produce energy to carry out their complex activities. This will create new opportunities to test new therapies that can stop inflammation, brain damage, and the subsequent development of neuropsychiatric disorders in the offspring.

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

The main output of this project will be new information on the mechanisms driving MIA in the mother that lead to brain inflammation and damage in the offspring. Additional outputs will include the publication of data from experiments in scientific journals. Datasets containing a large amount of information will be made accessible. We will use these datasets to apply for funds for future projects. These datasets will also be useful for other scientists and doctors studying neuropsychiatric disorders. Additional products from this project will be (1) tools to study immune and brain cell function or deliver therapies, and (2) technologies to identify if therapies are effective.

Ultimately, our approach will lead to the identification of new and important interactions of the brain and immune system. We can then create new methods to target these interactions and modify current approach to pregnancy to prevent neuropsychiatric disorders in the offspring later in life.

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

In the short term, the main beneficiaries of this project's outputs will be scientists from academic institutions and from pharmaceutical companies. In the medium term, the outputs generated by this project will help the NHS and the patient community. These outputs will advance the prevention and understanding of neuropsychiatric disorders. In the long term, these outputs will help us to create new tools that can be used to predict the risk of developing neuropsychiatric disorders in children and adults later in life by studying the metabolism of immune cells. In addition, we will better understand how to prevent the damage and brain reorganisation caused by MIA. This will lead to the identification of new targets that in the long term will be possibly modulated via specific therapies (e.g., anti-inflammatory) and/or dietary changes in pregnant mothers.

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

The outputs of this work will be distributed to academic scientists throughout the duration of the licence. We will communicate regularly with organised research networks and laboratory groups in the field of neuropsychiatric disorders and beyond. These networks will allow us to share workloads and ideas, but also to avoid repeated experiments, thus accelerating the progress in these fields.

Preliminary data will be shared at national and international conferences and/or workshops to gain valuable feedback from peers. This will also provide us the opportunity to build new collaborations locally and internationally, thus improving the quality and rigor of our

research for the duration of the licence. We will also present our findings ahead of publication on preprint servers.

When finalised, all our data will be published in peer-reviewed scientific journals. This data will include gene, protein, and metabolic datasets. We will also make these datasets available on appropriate databases for other research groups to access freely.
We are committed to publishing both positive and negative results. This will increase awareness and inform the community of how our findings fit into the wider field of study. It will also help in determining which experimental outputs are worth pursuing further and those we feel would not be worth further exploration.

Finally, we will ensure that the published results are made available to the general public. This will maximize their impact and increase global awareness to both the public and fellow scientists.

Species and numbers of animals expected to be used

- Mice: 1,340

Predicted harms

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

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

We are using pregnant female mice because currently they are the most used animal model of human maternal immune activation research. It is indeed extremely difficult to model the complexity of maternal-offspring interactions during pregnancy using cells in a dish. Therefore, using mice we can study many of the changes caused by maternal immune activation in the offspring. These include the presence of inflammatory immune cells, changes to the brain cells and blood vessels. Additionally, the possibility to use genetically modified mice to target DM is particularly important, as this will allow to study the role of DM in maternal immune activation and possibly identify a new therapeutic target (or biological readout) to prevent neuropsychiatric disorders in children and adults later in life.

Since ASD are more common in men than women, some of our studies on the offspring will reflect this. Here, we will use an increased proportion of male mice to female mice in our long-term experimental assessment of the offspring. Additionally, we will also use both young and old mice (up to 6 months) as controls to document the role of the alterations induced by maternal immune activation vs infection and dietary changes occurring later in life.

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

Mice will be subjected to maternal immune activation induction via either exposure to Polyinosinic:polycytidylic acid (Poly(I:C)) or exposure to a high fat diet (HFD) during pregnancy.

Poly(I:C) is a chemical compound made of RNA that is used to simulate viral infections as it is very similar to the genetic material found often in viruses (i.e., double-stranded RNA).

To model the Poly(I:C)-induced maternal immune activation, one or two female mice will be first paired with a male. At 12-16 hrs after mating, researchers will inspect the females for a vaginal plug (a gelatinous secretion deposited by a male into a female genital tract, such as the vagina, after successful mating). When a plug is seen, female mice will be weighted. Nine days afterwards, pregnant female mice will be weighted again and, if found to have gained >1.7g from their weight, they will receive one single injection of Poly(I:C) (or a control non-toxic solution) within the peritoneal cavity (the area that contains the abdominal organs). Pregnant female mice will be monitored for general health and weight twice a day in the 24hrs post-injection and afterwards on daily basis for a week. After birth, the male will be either killed or kept for future breeding. The females that received the Poly(I:C) injection will be killed after weaning of the litter, while the offspring will be kept until the end of the experiment.

The western high fat diet (HFD) is a special diet that has a high content of fat (60%, compared to normal diet that contains only 10%), which is used to study diet-induced obesity. To model the HFD- induced maternal immune activation, female mice will be given the experimental HFD diet (or experimental control ketogenic, or control normal diet) starting 4 weeks before mating. In the first 2 weeks, they will receive a 50%-50% mixture of HFD and normal diet. Mice will be checked on daily basis for general health and weight during the 1st week, and 3 times during the 2nd week. Female mice will be then given only HFD and checked on daily basis for the 1st week and then 3 times during the 2nd week. After these 4 weeks, one or two female mice will be paired with a male and kept on HFD (or control diets) until the birth of the litter. After birth, the male and the females will be either killed or kept for future breeding (in case of females, mice can be used again as breeders in protocol 2 of this PPL for a maximum of 3 litters). The offspring will be kept until the end of the experiment.

The study lengths for both maternal immune activation models may be as short as 21 days or as long as 6 months (maximum limit) to study both early and delayed inflammatory responses. Age and sex matched adult mice obtained from conventional breeding (i.e., without maternal immune activation) and exposed to either one single injection of Poly(I:C) within the peritoneal cavity or experimental diet can be used as further controls. Blood can be collected from these control and experimental mice using a superficial vessel during the experiment (once a week). The duration of each experiment is determined prior to the use of any mouse study and is variable in length. At the end of the experiment the offspring will be humanely killed, and tissues and organs collected. Tissue and organs collected this way will be stored in a solution that preserves their structure until follow-up analyses.

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

Within the Poly(I:C)-induced maternal immune activation protocol, mice are expected to display some signs of pain after injection, but this resolves quickly. Since this is an experimental modelling of a viral infection, the female mice are expected to develop symptoms within 24-48 hrs post injection, with a peak sickness response within the first 24hrs post-injection followed by a progressive improvement of health status within a week. Therefore, all pregnant female mice will be monitored for general health and weight at the time of injection and twice a day in the first 24hrs. After the first 24hrs, monitoring will depend on the symptoms mice display. These symptoms are classified as:

“No symptoms”.

“Stage I”, which are mild and expected. Stage I signs/symptoms include hunched posture, raised hair on the back, reduced activity compared to normal mice, weight loss (<10% from pre-injection weight).

“Stage II”, which indicates moderate signs/symptoms. Stage II signs/symptoms include dehydration, significantly reduced activity, repeated coughing or sneezing, diarrhoea, blood or discharge from vulva, weight loss (>10% but <15% from pre-injection weight).

“Stage III”, which are severe signs/symptoms that will require killing the female immediately. Stage III include coldness to the touch, continuous open-mouthed breathing for at least 5 minutes, immobility, vaginal or uterine prolapse lasting more than 1 hr, difficult birth (dystocia) lasting more than 1 hr, more than one generalized (“grand mal”) seizures, weight loss (>15% from pre-injection weight).

The high fat diet (HFD)-induced maternal immune activation is associated with an increased risk of depressive-like behaviours and obesity. Due to the disruption of normal feeding patterns in the initial first week of a full HFD introduction, mice can lose weight. Therefore, to prevent dietary change impact shock, experimental mice will be eased into this special diet starting 4 weeks prior mating.

During pregnancy HFD is usually tolerated, but it can increase the size of the foetuses. This can lead to difficult birth (dystocia) or vaginal/uterine prolapse after birth. In case of difficult birth, female mice will be given maximum 1 hr to complete the delivery, if this does not happen, mice will be culled humanely. In case of vaginal/ uterine prolapse after birth, female mice will be given maximum 1 hr to heal, if this does not resolve, mice will be culled humanely.

For both the Poly(I:C)-induced maternal immune activation and the high fat diet (HFD)-induced maternal immune activation experimental and control newborn mice are supposed to develop normally and have a normal life span after birth. In these mice, collection of blood using a superficial vessel will only lead to temporary discomfort for the mouse (equivalent of a very fine needle jab). The majority (>90%) of these mice remain healthy up to 6 months of age.

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)?

Mouse: Moderate 90%

Mouse: Severe 10%

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

- Killed

A retrospective assessment of these predicted harms will be due by 20 January 2029

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?

Neuropsychiatric disorders have received increasing attention from researchers during the past decades. The burden of disease is substantial, with more than 164 million people in the EU alone suffering from these diseases.

The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2019 showed that the most disabling mental disorder is depression, ranked among the top 25 leading causes of burden worldwide in 2019. While depression can occur at any age, ASD are the most common neuropsychiatric disorders of the childhood. it is estimated that worldwide about one in 100 children has ASD. This estimate represents an average figure, and reported prevalence varies substantially across studies. Some well- controlled studies have, however, reported figures that are substantially higher. Little was known about the causes of these disorders, until recent evidence suggested that events occurring during pregnancy can later cause neuropsychiatric disorders in the offspring. These events include stresses and stimuli like infections and inflammation that increase the activity of the brain immune cells (microglia) in the foetal brains.

Given the complexity of the mother-foetal interactions, the use of an animal experimental model in mice is necessary in this project to examine in a longitudinal manner the cellular and molecular mechanisms that underlie the normal developmental period, onset, and progression of neuropsychiatric disorders, as well as treatments promoting beneficial microglial activity. In fact, complete in vitro approaches to model these aspects do not exist. The choice of the mouse model is justified not only by the excellence of the experimental model, but also by the need to use transgenic mice that can be used to remove certain cells (like "dark microglia") from the brain. This will be important to identify new players and targets for treatment.

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

Over the years our team has refined and improved upon our models of cells grown in lab culture dishes. This has allowed us to test whether treatments will be (1) safe for cells and (2) how effective the treatments are before testing them in a mouse. Additionally, we have developed a new model of maintaining and expanding human immune cells and human stem cells in plastic dishes that does not involve the use of mice.

This new system allows us to (1) capture the response of human cells in a dish and (2) design/perform experiments to test ideas about the response of cells without having to extract these cells from mice beforehand. We also investigated previously published gene expression datasets (RNA sequencing) maternal immune activation models, but single cell RNA sequencing approaches at different stages of maternal immune activation are lacking.

Why were they not suitable?

Immune cells grown in lab culture dishes are useful for studying some aspects of immune activation. However, they cannot replicate the complex changes that occur in the cells and tissues of a living organism, nor the interactions between the pregnant mother and the offspring.

These include changes to the cells of the brain and spinal cord (neurons) and to the immune cells of the brain (microglia). Immune cells and neurons grown in culture also behave differently to those found in a living organism, as they show loss of cellular heterogeneity (i.e., the unique identity of individual cells) and loss of communication with other cell types.

It is therefore necessary to use animal models. This allows us to assess the complexity of biological and behavioural responses throughout the animal lifespan.

A retrospective assessment of replacement will be due by 20 January 2029

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?

Mouse numbers were estimated based on data reported in the literature and data provided from our collaborators. With the new mouse colony management system in use, we now have access to our total mouse usage year-over-year.

For the Poly(I:C)-induced MIA protocol, we are expecting to use n=120 female mice for pregnancy/breeding. With an average of 5 mice per litter, this will lead to 300 offspring. Control adult mice will be 200. Over the course of this five-year licence we will use an estimated total of 620 mice in this experimental protocol.

For the HFD-induced MIA protocol, we are expecting to use n=120 female mice for pregnancy/breeding. With an average of 5 mice per litter, this will lead to 300 offspring. Control adult mice will be 300. Over the course of this five-year licence we will use an estimated total of 720 mice in this experimental protocol.

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

The National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs) experimental design assistant is a tool which we constantly use to help design and further refine our experiments. We also reference the Planning Research and

Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines.

According to our lab standard operating procedures (SOPs), experiments are constantly assessed at the pilot stage first. This is when a first experiment is conducted with a reduced number of mice to adjust any aspects before running the full experiment. This guarantees that we are using the correct number of mice to achieve reliable statistical results when experiments are ready to be conducted in full. Mice are then placed in the experimental groups randomly, which helps to ensure treatment and non-treatment groups are evenly distributed. Treatments are given 'blind'. This means that the person giving the treatment (or a vehicle, as control) have been given no access to the information related to the treatment they are giving. Unblinding (i.e., informing the scientists of the treatment given) is the responsibility of the principal investigator and it is done only after the experiment is concluded and results are analysed. This is to avoid any bias in the generation of the results.

Specifically for female mice in Protocol 1, we noted that the presence of a plug (i.e., a gelatinous substance that covers the entry of the female reproductive tract after mating) is not a definitive indicator of true pregnancy, particularly in inbred mice (in which false-pregnancy rates have been reported to be 50% or higher). This could mean that female mice may receive the Poly(I:C) injection even if they are not pregnant. To reduce this occurrence, we have implemented our approach by using data from literature showing that if female mice are found to have >1.7g weight gain in the first 10 days of pregnancy, the false-positive rate (i.e., the rate of false pregnancies) is reduced to 10.5%, without excluding any pregnant mice. Thus, we have included a threshold of 1.7 g weight gain at day 9 in our mice, as a further check before giving the poly (i:c) injection.

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 always perform pilot studies before undertaking a full experiment to ensure that larger studies are as accurate as possible. These pilot studies allow us to assess the experimental design and identify potential problems, as well as implement improvements early on in the licence. We are also coordinating with other groups to share animal tissues - including tissues from genetically modified mouse lines and post-mortem tissues - to further reduce overall mouse numbers.

A retrospective assessment of reduction will be due by 20 January 2029

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 maternal immune activation hypothesis proposes that inflammation in utero can affect fetal neurodevelopment, and evidence from human epidemiological studies supports an association between maternal inflammation during pregnancy and offspring neurodevelopmental disorders. Rodent models of maternal immune activation are increasingly used as experimental tools to study these neuronal and behavioural dysfunctions in relation to neurodevelopmental disorders.

One of the most widely used maternal immune activation models is based on gestational administration of Poly(I:C), a synthetic analogue of the genetic material found often in viruses (double- stranded RNA). This challenge induces a viral-like acute phase response that causes neuropathological alterations in the offspring. Another most widely used maternal immune activation model is based on changing maternal nutrition, which is critical for proper fetal development. While increased nutrient intake is essential during pregnancy, an excessive consumption of certain nutrients, like fat, can lead to long-lasting detrimental consequences on the offspring. Animal work investigating the consequences of HFD revealed in the offspring a maternal immune activation phenotype associated with increased inflammatory signals.

While invertebrate organisms share some elements of metabolic control with humans, to obtain meaningful data we must use mammalian models of maternal immune activation. Rodents have the enormous advantage of being readily susceptible to genetic manipulation enabling precise alteration in the function/expression of specific genes and the creation of animal models of relevant human diseases. In addition, the complexity of the mammalian brain is key to model central metabolic-sensing circuits, and this research question cannot be addressed in lower organisms.

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

We are extremely limited in the use of invertebrates (e.g., worms), fish, or amphibia. These animals are not fully suitable for the development and testing of new therapeutic targets for humans with neuropsychiatric disorders. Some preliminary work on regenerative biology can be done in non- mammalian species. However, the complexity of the interactions between the maternal and foetal immune system and the brain in the context of infection and diet can only be studied in mammals. This is because they possess a body structure with similarities to the human central nervous system and immune system. We also cannot rely completely on animals that have been terminally anaesthetised as we need to produce offspring from the pregnant females that will undergo treatment.

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

To cause the least pain, suffering, distress, or lasting harm to the animals, we will adopt several refinement steps, as follows:

Minimising suffering. In designing experiments, we use standard operating procedures and non- invasive techniques whenever possible to keep suffering to a minimum. Early detection of side effects to prevent animals’ suffering will be performed and analgesics will be used when appropriate.

Improving environmental factors. When using single housing, we always add old bedding to maintain the olfactory environment, add old standard nesting and enrichment material, and keep the old cage for when the animals return to group housing if appropriate. We will regroup mice whenever possible after short term single housing sessions. In these cases, we will monitor for potential aggressive behaviours.
Refining the use of gene inducing agent (Protocol 2 only). We are optimising the use of gene inducing agents to avoid side effects and increase efficiency using pilot studies and trying ways to limit weight loss (supplementation with diet mash during tamoxifen administration).

Welfare assessments. Weight loss will be used to assess welfare in the majority of the cases. However, when physiological response occurs in response to scientific procedures, weight loss is not an indicator of well-being. In mice presenting weight loss, we will always determine whether the presence of other clinical signs justifies the interruption of the experiment and seek the advice of the NVS to avoid unnecessary animal waste.

Further refinements will include increased monitoring and pain management.

Before starting any study plan, we will discuss all experimental methods with the appropriate staff within the animal unit. This will guarantee that all the necessary equipment is in place to perform procedures under optimal conditions and/or supervision. This is to maintain the best health and welfare of the animals. Prior to running studies, we will determine if the necessary staff and expertise is available to successfully run the whole study. This way we can ensure that no skills are missing to guarantee the study is successful and that relevant equipment is available to process samples under optimal conditions.

Once the study has started, we will rely on our established step-by-step care packages. This will minimise the harm to the mice and ensure that the welfare of the mice is never compromised.

Refinements will be centred around the housing of mice experiencing expected adverse effects and to the daily care and monitoring of the mice, which include the following: (1) providing bedding that does not inhibit the free movement of these mice, (2) heating pads fixed to the bottom of the cages to maintain stable core body temperature, (3) placing wet mashed food on the cage floor to encourage eating and allow ease of access to disabled mice, and (4) providing cardboard houses.

If needed, we will also use pain medications to ease disease complications (after NVS approval), and perform fluid replacement through subcutaneous (i.e., under the skin) injections if dehydration is present. As part of our daily monitoring, mice will have their abdomen checked by an experienced user to identify signs of infection or dystocia.

Finally, full training will be provided to new technicians who are unfamiliar with these procedures. This helps new technicians to learn how to assess our mice correctly. This guarantees that mice recovering in our experiments receive the same high quality and consistent level of monitoring and care they need.

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

We plan our experiments in accordance with the guidance provided in the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines. This will guarantee we use the minimal number of animals to answer our objectives and ensure our results are both robust and reproducible. We will follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines when preparing our data for publication. In so doing, we will ensure our published findings are complete and clearly presented and easily accessible to other groups. This will lead to a reduction in the unnecessary duplication of animal experiments.

Excellent information is available on our establishment website, which is routinely updated with new 3Rs information. The NC3Rs website will be regularly consulted to be sure that we are applying the latest recommendations for the refinement of our experiments. We will also consider any new publications in a peer-reviewed journal relevant to our field offering refinements to our protocols.

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

Our establishment offers continuous training and recommendations via the animal facility and from animal care staff located within. We will keep informed of any changes to animal welfare guidelines by regularly consulting the website they provide. This will ensure that we maintain compliance should any new updates be posted.

The NC3Rs will be the main reference to assess whether our experiments match the highest standards of 3Rs. We will adapt our protocols if the recommendations evolve throughout the duration of this project. Regular consultations on the latest practical guidance from Laboratory Animal Science Association (LASA), Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) will provide additional sources of new recommendations and advances in animal techniques and clinically applicable models.

Training records for all personal licence holders will be kept up to date using a centralised database. Senior group members will provide extensive training on the relevant regulated procedures to all new lab members who will be working with animals. Further, new lab members will be informed of the mandatory training services available to them. This will guarantee that general practices are firmly adhered to and will ensure the welfare of the animals is consistently upheld. As a licence holder, it is my own responsibility to stay updated on published best practices. This will be done by consulting information for licence-holders provided by our establishment and by speaking to other project licence holders.

A retrospective assessment of refinement will be due by 20 January 2029

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. Production of biological samples

Project duration

5 years 0 months

Project purpose

- Basic research
- Development, 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

Blood, Urine, Biological

Animal types	Life stages
Mice	adult
Rats	adult
Rabbits	adult
Beagles	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 purpose of this licence is to acquire blood and/or urine samples from animals for subsequent use on *in-vitro* studies whereby, the data generated can then be used to assist with the interpretation of results from regulatory and/or non-regulatory studies.

In addition to this, the biological samples taken can be used to validate and or calibrate laboratory equipment before they are used on regulatory and or non-regulatory studies thereby, ensuring that they are suitable for purpose and can generate accurate data consistently.

The aims of the project will be achieved by undertaking one or more Steps as detailed in the Protocol. Due to regulatory requirements the Steps will have to be optional.

A retrospective assessment of these aims will be due by 21 January 2029

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 majority of studies performed for regulatory purposes are run to Good Laboratory Practice (GLP) standards and therefore, the laboratory equipment used to generate study related data must validates as suitable for purpose and are capable of generating accurate data consistently; for example, equipment used to analyse changes in blood and or urine parameters for example . The studies performed under this licence will allow us to run validation studies using the biological samples obtained thus enabling us to assess the suitability of equipment before being used on valuable studies; and similarly, where necessary, allow us to calibrate the equipment before being used.

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

The biological samples collected will allow data generated from *in-vitro* studies to be assessed thereby, increasing our understanding of pharmacological processes such as metabolism, protein binding and bio-availability of a drug. These data will help to guide subsequent, *in-vivo* toxicity studies, ensuring they are conducted at appropriate doses and in relevant species.

Control matrix will also be provided to support bioanalytical (pharmacokinetic and toxicokinetic) analysis in other licenced projects.

The validation and calibration of equipment and the establishment of methodology prior to use on future regulatory and non-regulatory studies will allow data to be collected in a GLP compliant manner which is acceptable to International regulators.

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

Whilst laboratory equipment is typically designed to be used in a particular way, different Establishments will tend to perform their own validation studies and not share data with external colleagues due to the fact that any minor changes in the operation of the equipment may affect the integrity of the results. The outputs of work performed under this licence will tend therefore, to benefit our Establishment only and will be seen in the short-term i.e. within a few months.

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

The outputs of this work (the generation of data which allows the validation of laboratory equipment and an assessment of whether they are suitable for use on regulatory safety studies performed to Good Laboratory Practice standards) cannot be shared with other establishments as they will prefer to undertake their own evaluation studies due to the fact that any differences in the way they use the same equipment may generate different result. The data will be maximised however, by using data from one study to establish the suitability of similar equipment used at this establishment; thus negating the need to perform additional studies using more animals.

Species and numbers of animals expected to be used

- Mice: 200
- Rats: 400
- Rabbits: 50
- Beagles: 10
- Minipigs: 50

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 purpose of this licence is to support the performance of regulatory studies which use common laboratory animals i.e. rat, mouse, rabbit, minipig and where scientifically justified, the beagle dog. For this reason, it is essential that the same species are used in order to make any data generated relevant and useable.

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

The procedures performed will be the acquisition of blood and/or urine using well established techniques.

In the majority of cases, blood will be obtained from superficial blood vessels, for example the marginal ear vein in rabbits, the tail vein in rodents, the cephalic or saphenous vein in dogs (leg veins). With regards to blood sampling in minipigs, typically the marginal ear vein or the saphenous vein is used, but where a 'larger' volume is required then we may use the vena cava.

Blood samples will typically be taken on one occasion only, but their may be occasions where multiple samples are required in a 24 hour period (no more than five samples)

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

It is expected that animals will experience, mild, transient levels of pain or discomfort only.

Blood samples will be taken using well established routes, typically from superficial vessels (*). The volumes taken will be within acceptable limits as detailed in industry

accepted guidance documents, for example "A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes", Karl-Heinz Diehl etc. (*) There will be circumstances whereby, a larger volume than those detailed guidance document is required and under those circumstances a terminal blood sample may be taken from an animal under deep, general anaesthesia without recovery. This will ensure that the animals will experience no pain, suffering or distress during the procedure.

Urine samples will be taken from small rodents (rats and mice) by placing them into metabolism cages for a defined period. This may cause mild levels of transient distress until such time the animals become accustomed to the new environment. With regards, to urine sampling beagle dogs, the dog will be appropriately restrained, typically by holding and a catheter introduced into the urethra and into the bladder. This procedure will be performed under clean conditions to minimise of introducing an infection into the urethra. Once again, the levels of discomfort will be transient and mild. Where considered necessary, the use of a local anaesthetic may be used in order to reduce discomfort. Urine samples will not be taken from the minipig or the rabbit.

The procedures performed are not expected to adversely affect the health an well-being of 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)?

Previous experience gained from taking biological samples from laboratory animals gives confidence that 100% of all animals used will experience, mild, transient pain or discomfort effects only.

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 21 January 2029

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 primary purpose of this licence is to obtain biological samples i.e. blood and urine to support in- vitro studies which require these products as part of establishing and validating new methodologies and, calibration equipment. At this time there are no suitable

substitutes for blood or urine that are internationally accepted and therefore, it is still a necessity to acquire biological fluids from animals.

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

Non-animal alternatives have not been considered for this project.

Why were they not suitable?

The use of non-animal alternatives will not allow the objectives of this licence to be fulfilled as the primary purpose of each study will be to use blood and/or urine samples to validate and/or calibrate laboratory equipment, or to perform *in-vitro* studies where said biological samples are required.

A retrospective assessment of replacement will be due by 21 January 2029

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 particular application will replace a licence with similar objectives and therefore, the experience gained from performing studies under that licence will be used to determine how many animals of each species will be required to support study objectives.

In addition to this, there may be occasions where a specific quantity of biological sample is needed for a given project, and on such occasions the number of animals required will be calculated by reference to blood circulatory volumes as determined by industry accepted, good practice guidelines versus the number of samples that can be taken in a given period.

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

Regulatory studies (performed under separate authority) are designed using the minimum number of animals practicable to achieve their objectives and in specific circumstances the numbers required are determined by legislation. If an animal sustains an injury, or its health status is of concern before the study starts, or in the first few days of study commencement an animal needs to be euthanized on welfare grounds then the integrity of the study could be compromised because of insufficient numbers of animals available. For this reason it is often prudent to obtain more animals than is required so that if an animal does need replacing, it can be done so using an animal of similar specifications.

Unfortunately, this does mean that when these additional animals are not required and there are no suitable studies for them to be transferred to, they would typically be euthanized. The work conducted under this licence however, avoids animal wastage by allowing them to be used in an ethical and worthwhile manner.

On the rare occasions when biological samples are required but animals are not available from stock, we may choose to acquire animals from a designated supplier; but before we do, we will always consider using animals from other project licences that have been approved for re-use. If such animals are available and deemed suitable for purpose then this would negate the need to acquire additional animals thus reducing the number of animals that would otherwise have been used further.

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

The primary use of animals used in this licence is to acquire blood and or urine samples for the validation and/or calibration of laboratory equipment or for use on other *in-vitro* studies. There will be occasions where multiple biological samples are required over several time points covering several days or weeks, and on these occasions samples will be taken at preselected times, the volumes of which will be in-keeping with established, good practice guidelines. A final sample however, may be taken under deep, terminal anaesthesia which allows larger volumes to be taken without causing the animals pain, suffering or distress; these larger volumes can be frozen and banked for future use. Such a process will optimise the numbers of animals used during the life of this licence as well as significantly reducing the numbers of animals that would otherwise have been used.

A retrospective assessment of reduction will be due by 21 January 2029

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 procedures used for taking blood and urine samples from animals used on this licence (rat, mouse, rabbit, minipig and the beagle dog) are well established and refined and will cause transient discomfort or distress only.

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

The species chosen i.e. the rat, mouse, rabbit, minipig and the beagle dog have been

selected because any data generated from studies using the biological samples obtained from these animals will be used to support regulatory safety studies using these species.

There will be occasions when animals will be terminally anaesthetised in order to obtain a blood sample; however, because of the mild severity of this procedure it may be more ethical to use the animals for further sampling instead of killing the animals and acquiring additional animals.

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

The refinement of procedures will be undertaken under separate authority. We have at our disposal a project licence that enables method development and refinement of regulatory procedures with the aim of reducing severity and minimising welfare costs for the animals.

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

Blood samples will be taken using well established sampling techniques, some of which will have been refined at our Establishment under separate authority using a licence specificially for the purpose of method development and refinement of techniquies. In order to ensure good animal welfare, the volumes of blood taken will be in compliance with good practice standards as detailed in "A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes", Karl-Heinz Diehl etc.

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

The Establishment has key members of staff that are members of various, scientific Forums that discuss advancements in study design, animal welfare, including enrichment, and advances in regulatory procedures. These will then be discussed internally and introduced as necessary.

A retrospective assessment of refinement will be due by 21 January 2029

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. Toxicity of pharmaceuticals and medical devices in the rabbit

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Rabbit, Toxicology, Irritancy, Pharmaceutical, Medical device

Animal types	Life stages
Rabbits	adult

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- 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 determine scientific and/or regulatory endpoints in rabbit toxicity, including immunotoxicity, toxicokinetics / toxicodynamics / biodistribution / persistence / biomarker and supplementary/investigative toxicity, tolerance and/or safety. The test materials under investigation will be human or animal pharmaceuticals (and pharmaceutical intermediates) / biopharmaceuticals / biologics, medical devices (and components of medical devices) and drug delivery systems.

No cosmetic products or chemicals that are exclusively intended to be used as ingredients in cosmetics will be tested.

A retrospective assessment of these aims will be due by 26 January 2029

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?

Governmental approval is required before a new pharmaceutical or medical device (or medical device component)may enter clinical human or veterinary trials. further approval may also be needed before it can be marketed. There is an expectation, both governmental and public, that these materials should be safe for use, or that their potential hazards are well understood and documented to enable appropriate risk-based assessments and regulatory decisions to be made.

Guidelines issued by government appointed regulatory agencies specify the types of animal and non- animal studies that must be completed in order to apply for clinical trial authorisation or product marketing approval. These guidelines routinely require studies to be performed that will assess general systemic, developmental and reproductive toxicity, mutagenicity and tumorigenicity of the pharmaceutical or medical device (or medical device component) that is being developed. This project licence focusses on general toxicity, local tolerance and irritancy studies in the rabbit.

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

The overall benefit of this project is that it supports the development of safe, new medicinal products and medical devices to improve the health and quality of life of human and veterinary patients by generating high quality data that is acceptable to regulatory authorities and enables internal decision making within our clients' organisations. This may lead to new and improved treatments for common diseases and ailments.

Achievement of the objectives of this licence will enable safe drug development candidates and medical devices to progress and will also help to remove unsuitable candidates from the development pipeline at an early stage, thus saving animals and resources.

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 medicinal products/medical devices under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain clinical trial approval or marketing authorisation.

Patients and animals will benefit from these studies as this work will contribute to the development of new drugs that help alleviate human and conditions. 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. Similarly for medical devices, they may be better than existing devices or a new device which can improve patient outcomes (examples of medical devices include artificial hip replacements and dressings for wounds).

The toxicity information obtained is important when planning future trials in humans and animals, to make sure any starting dose in a clinical trial is safe for the participants taking it.

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 regulatory purposes or to support regulatory purposes (e.g. to support drugs/devices progressing to clinical trials). Previously however, we have collaborated with customers and shared data we have produced in the form of scientific publications that are in the public domain.

Species and numbers of animals expected to be used

- Rabbits: 2850

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.

Regulatory authorities require the initial use of rodents and a second, non-rodent, species; the rabbit is often employed as the non-rodent species of choice. The rabbit behaves similarly to humans following exposure to irritant substances and as such they are used on studies that look for irritant effects of a test item. Since the size of the rabbit allows the human dose of a vaccine to be administered, they are used on studies to assess the efficacy and safety of vaccines (for example).

Adult animals are used throughout this project.

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

Substances will be administered to the animals by standard routes of administration such as injection (into the blood, into the muscle or under the skin), direct application to the skin or eye or oral (gavage). For pharmaceuticals, the usual route of administration is the proposed clinical route. Some substances may be surgically implanted (such as medical devices) and for others, devices may be implanted to deliver test substances slowly over a period of time.

Animals will be anaesthetised for surgery and for other procedures when considered necessary (such as for the placement of an access port into a blood vessel to allow blood samples to be taken avoiding the need for multiple injections , or directly administer

substances into tissues such as bones, sinuses or eyes). Veterinary surgeons are consulted prior to these studies starting, and pain relief and antibiotics are administered, similar to a patient undergoing surgery in hospital. Aeseptic techniques are applied to surgeries. When dosing an animal by injection or taking blood, the degree of pain or discomfort an animal feels is similar to what a patient would feel having an injection done by a doctor

Animals may be restrained (either held by the technicians or by wearing bandages, jackets, Elizabethan collars or stocks) to aid administration of substances or to prevent the animal interfering with the administration site.

At the end of the study, the animals are normally humanely killed and subjected to post mortem examination with tissue samples being taken for analysis.

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

Whilst on study, the animals may experience reactions to treatment such as decreased activity, changes in posture or gait, decreased appetite, weight loss, breathing irregularities and, in some circumstances, the animal may die. Whilst death is not the aim of any of our studies the test substances used may have unknown effect on animals. We endeavour to avoid death due to toxicity by using small test groups initially to aid in setting dose levels for main studies and if no data are otherwise available for the particular test compound we may extrapolate from other species where the same compounds has been used or from other rabbit studies using similar compounds if such data are available. For all studies we closely monitor animals and if predetermined humane endpoints are reached based on signs observed we humanely kill to avoid unnecessary suffering.

Experience shows that around 80% of animals show subtle or mild signs (such as subdued behaviour); moderate signs (such as 15% weight loss) may be seen in around 20% of animals, usually in the high dose groups. A very few animals (<1%) may show severe signs (such as fitting). Most dosing techniques, manipulations or investigations do not cause lasting adverse effects, but a small number of animals may show transient mild or moderate distress (such as withdrawal of blood or administration of a mydriatic).

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 numbers from the last project around 80% of animals would be expected to show mild clinical signs, and 20% of animals moderate signs (high dose groups or had a surgical procedure).

It is impossible to predict the proportion of severities expected on a service licence like this, as this will be dependent on what study types we are asked to perform, however, a distribution between ‘mild’ and ‘moderate’ severities similar to those in the last project are anticipated.

Although there are severe severity procedures on this project, on the last project less than 1% of animals experienced severe clinical signs.

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 26 January 2029

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?

Although non-animal (in a test tube (in vitro) or computer modelling (in silico)) studies can provide useful supporting data to refine and reduce animal studies, definitive assessments of whole body or 'systemic' exposure, efficacy and toxicity can only be achieved in studies using intact animals.

This is because each body system does not act alone but is part of an overall interrelated biological system with influences from other organs within that system. To date it is not possible to model a whole body system in the laboratory and the use of animals in regulatory toxicology, and this remains a mandatory legal requirement.

We will, however, remain vigilant to seeking alternatives where possible and where such tests are available, use validated non animal alternatives (for example to screen out, using cells in a dish rather than a whole animal, substances that are shown to be corrosive such that work does not further progress into animals.
Currently, however, for many of the study types within this project, there is unfortunately no scientific, ethically or legally accepted non-animal alternative 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?

There is no other non-animal alternative 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.

No study in animals is conducted under this licence until an assessment has been made to determine that the specific study is necessary and justified, i.e. the study aims and objectives are consistent with the scope and purpose of the licence and cannot be achieved by any other means not involving the use of animals. This assessment will involve consideration of any potential non-animal alternatives, review of existing data on the test item and reference to any other relevant information (including literature review, in-house data, information on similar items).

Why were they not suitable?

Although there are in vitro tests that can model some parts of how drugs get into our bodies, and how our body deals with them, and can identify undesirable effects, for example, there is no series of in vitro tests that brings all these complex events together, as in the whole (animal or human) organism.

That is why we need to test new drugs in animals, as they have similar physiology and processes as humans, and that testing gives us a good idea what may happen if they are subsequently used in humans. For animal pharmaceuticals, the use of the target species is mandatory.

A retrospective assessment of replacement will be due by 26 January 2029

The PPL holder will be required to disclose:

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

Reduction

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

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

The numbers we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received from customers in the past. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

The numbers of animals used in each study are in some cases specified in the regulatory guidelines; where not specified, numbers are based on established minimum regulatory expectation, or on scientific estimates of the minimum numbers required to meet study objectives.

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.

For regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international guidelines where these exist. Otherwise reference is made to standard study designs with input from the Department of Statistics, where appropriate, to identify the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. These internal designs are reviewed and updated in line with changing external guidelines and internal refinements that either minimise numbers or reduce severity.

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.

Before we embark on animal testing we ensure that we have all the relevant data to hand either from early phase studies that either we ourselves have completed (or are available from the sponsor) or from seeking further information in literature. We take a staged approach to testing such that if a compound is shown to be unlikely to be effective or shows unacceptable toxicity via proposed route of administration it can be removed from the testing programme at an early stage and not further progressed thus avoiding 'waste' of animals.

For later phase studies, the numbers of animals used are kept to the minimum that would comply with 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?

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, we use smaller groups of animals to get an idea of the doses (of pharmaceuticals) we need to use for the main studies. 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. Preliminary studies are often used when testing medical devices.

A retrospective assessment of reduction will be due by 26 January 2029

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 uses adult rabbits

For studies that assess the effect of a test substance to cause irritation before the study starts we will undertake a weight-of-evidence analysis using all available information on the material. Such information will include chemical characteristics of the test substance and, where available, results from non-animal studies and computer modelling. The results of this analysis will be used to offer a prediction of the potential of the test item to cause a severe effect on an animal and the need for a live animal study. When acceptable to the regulatory agencies such studies will be conducted by an appropriate in vitro method.

Sequential testing, with review of findings at each stage and modification of subsequent stages as necessary, maximises opportunities for refinement to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals.

Animals are monitored for clinical signs of toxicity or other effects on their health and wellbeing, and in order to prevent unnecessary suffering, humane end-points are applied under appropriate veterinary guidance (e.g. modification/withdrawal of treatment with the test substance, provision of palliative or therapeutic treatments, or humane killing of affected animals).

The models we use are the least invasive procedures, for the least amount of time necessary to get the information we need. They are carried out using standard and recognised techniques. We also have veterinary clinicians on hand for advice and on the occasions we have to anaesthetise the animals and for general advice on animal welfare.

If we have to repeatedly inject animals or 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 place temporary cannulas in blood vessels to reduce the number of needle punctures necessary . If we can take blood samples when an animal is deeply unconscious then we do so.

Surgical procedures will be carried out aseptically and to at least the Home Office minimum standards for aseptic surgery, and in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) (LASA is the Laboratory Animal Science Association).

Animals in surgical studies are normally regarded as experiencing moderate adverse effects (though they are given appropriate pain relief medication) and may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients.

The rabbit is a naturally social species, living together in groups in the wild. Accordingly where possible, rather than house animals individually, we will (specifically with female animals who are less inclined to fight when housed in single sex groups than males) house female animals in social groups of two or three individuals.

Where more than one method of assessment exists, the least invasive method /most 'refined' method will be employed (for example for the recording of body temperature we will use a human/paediatric thermometer that can be held on ear of rabbit or subcutaneously implanted microchip transponder rather than using a thermometer than is inserted into the rectum (the use of a rectal thermometer requires justification to the PLH, NVS and NACWO).

Refinements under the previous programme of work PPL have included:

- the use of refined methods for the collection of body temperature (thermometer for use on ear and microchip transponder under skin as the primary methods.
- the twice weekly offering of a small quantity of vegetables as a dietary supplement rather than feeding of pelleted rabbit food alone.
- the group housing (normally in social groups of two or three) for female rabbits.
- the inclusion of shelves in all cages to provide 3D complexity to the cage, enabling an animal to explore the full height of cage or to rest in the area under the shelf if desired.

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

Regulatory authorities require the initial use of rodents and a second, non-rodent, species; the rabbit is often employed as the non-rodent species of choice (in preference sometimes to the dog or pig)

The rabbit behaves similarly to humans following exposure to irritant substances and as such they are used on studies that look for irritant effects of a test item. Since the size of the rabbit allows the human dose of a vaccine to be administered, they are used on studies to assess the efficacy and safety of vaccines.

Most of these studies require repeat dosing for days, weeks or months, to assess potential adverse effects in man, so 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?

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet at least the standards set out in the Home Office Minimum Standards for Aseptic Surgery. Before we start surgery, we agree with a Vet what pain killers or antibiotics the animals need both before and after the surgery. When animals are recovering from surgery, we give them extra heat and monitor them closely until they are fully recovered and showing normal behaviour. We then check them regularly before they go on study.

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, then

we would habituate animals to this equipment prior to dosing. Most animals habituate well to this equipment, but if they don't (rare) we remove them from the study.

If we have to repeatedly inject animals or 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 place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. If we can take blood samples when an animal is deeply unconscious then we do so. All personnel performing these procedures are trained to a high standard to minimise adverse effects.

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.

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

LASA (2017). Guiding principles for preparing for and undertaking aseptic surgery. A report by the LASA Education, Training, and Ethics Section (Eds. Jennings & Berdoy).

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

International Standard Organisation. Biocompatibility of medical devices. ISO 10993
EMA (1997). Preclinical pharmacological and toxicological testing of vaccines. CPMP/SWP/465/95

OECD ENV/JM/Mono (2007). Guidance document on the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation.

EMA (2015). Guidance on non-clinical local tolerance testing of medicinal products. EMA/CHMP/SWP/2145/2000

OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects

ICH M3(R2) (2009) Guidance on Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.

Preclinical pharmacological and toxicological testing of vaccines. EMEA CPMP/SWP/465/95

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.

A retrospective assessment of refinement will be due by 26 January 2029

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. Provision of an outsourced drug development platform for the treatment of bleeding disorders

Project duration

5 years 0 months

Project purpose

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

Key words

Bleeding disorders, Haemophilia, Thrombosis

Animal types	Life stages
Mice	adult
Rats	adult
Rabbits	adult

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- 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 licence is to provide a service to drug discovery clients to support the development of novel drugs for bleeding disorders that have an unmet clinical need.

As part of this work we will improve and refine animal models of disease to ensure they are fit for purpose and are the most appropriate to answer the scientific question when testing new drugs.

A retrospective assessment of these aims will be due by 30 January 2029

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?

Despite notable progress in the discovery and development of medicines, there is still a significant unmet need in the treatment of bleeding disorders, affecting 1 in 2000 people in the UK. Bleeding disorders can severely affect quality of life; life-long care including lengthy stays in hospital is not uncommon for patients. Bleeding disorders can have short- and long-term consequences, and treatments can become ineffective if the immune system fights against them. Finding treatments for all aspects of patient care therefore is crucial to improve outcomes for people affected.

This project will generate important proof-of-concept data in the development of potential new test agents (drugs) targeting bleeding disorders, ranging from the most common (haemophilia A), to rarer (e.g. Glanzmann thrombasthenia). These studies will generate vital information that cannot be found without the use of animals. All clients that we work with are developing test agents which will hopefully contribute to helping people with various bleeding disorders, where the need is greatest.

This licence forms part of a suite of project licences that cover the ability to test a potential therapeutic at the early stages; most relevant is a licence that allows us to determine the suitability of a compound (e.g. how it's tolerated, what dose is needed) in healthy animals first.

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

This project will provide important information that aids the progression of new drugs against bleeding disorders through the drug discovery and development process. The information gathered will enable us and our clients to identify the most appropriate treatments to take forward to human clinical trials and enable us to quickly determine which drugs should not be progressed any further.

In addition, this work will increase our knowledge of how new drugs work and will help us to identify changes in the body that occur in response to the drug. We can use this further down the line to monitor responses in humans during clinical trials.

Data from studies under this project may also be used to support patent applications and applications by clients for additional funding. Data produced may also support the design of regulatory studies for clients.

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

Bleeding disorders can impact patients in different ways; some of which can cause too

much clotting in the blood, others cause too little. For example, haemophilia patients have bleeding episodes, which can cause blood to gather in the joints and cause major pain. Haemophilia can even be life-threatening if blood loss from the circulation is too high, and prompt treatment is crucial. Other bleeding disorders can cause life-threatening blood clots to form that can cut off blood flow to different areas of the body.

Regardless of the kind of disorder, due to their nature they significantly impact on the quality of life for patients, and both protective and on-demand treatments are needed for most conditions. Current medications, however, can often fall short in being effective for some or all patients, create side effects that also impact on patient care, or require lengthy stays in hospital due to how they need to be given. Further work is needed to develop safe and effective medicines for a multitude of bleeding disorders, and tackle some of the challenges with treating them.

This programme of work is expected to enable us to progress new treatments for bleeding disorders through the key milestones of drug development. Drugs shown to be effective in this project can be advanced into the clinic for testing in patients where they could significantly improve a patient's quality of life.

In the short term benefits will be seen by the client; by the collection of data from programmes that will increase the knowledge of their lead candidates for proof-of-concept (is it possible to use this drug in animals), and efficacy (does the drug treat the disease). Additionally we will continue to increase our knowledge of the best ways to closely mimic the human bleeding disorders.

In the medium and long term, later-stage development of the drug (clinical trials) and if proven effective and safe when taken into the clinic, will see the work under this licence contribute to benefits seen directly by the patients.

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

All studies are designed such that the outputs from each animal are maximised. Expert knowledge is gathered not only from within the preclinical (animal) team performing the animal studies, but from other teams within our company, or our clients' companies. This ensures that all relevant work that has been performed in the laboratory is taken into consideration when designing animal studies. The in vitro (in the test tube) and bioanalysis teams at our company are experts at analysing tissue and blood samples collected from animals and they help with details of sample collection and storage to ensure that the samples are collected and stored in the best way possible. They are also experts at working with small samples, particularly small volumes of blood, meaning that they can often analyse lots of different biomarkers (a measurable indicator of a disease state or other physiological state) and test agent levels from each animal. Any tissues resulting from projects that can be utilised by other projects will be made available. Our company has a very comprehensive sample record system which makes it very easy to assess the tissues we have banked and the conditions of how they were collected and stored.

In addition, we will seek expertise from our established networks, to ensure that we make use of any new knowledge or incorporate better methods of performing animal studies. We will also use these networks to provide information and training to others on the models and techniques we use in our research. We will maintain good communication with managers of the animal facilities to ensure that any tissues from animals being killed that are not required for our work can be made available to other researchers if suitable.

Although there are times where we will not be able to share animal model information (for example, where it would put us at a competitive disadvantage), we aim to publish or share our findings wherever possible, such as control data or where notable refinements have been made in a disease model or procedure.

Species and numbers of animals expected to be used

- Mice: 4000
- Rats: 1750
- Rabbits: 310

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 are the most common type of animal used for generating 'models' of bleeding disorders and for the testing of new treatments. These models mimic areas of the disease in order to provide data on which drugs are likely to work well in humans as a treatment. The immune system of both species has been highly studied, meaning that a lot is already known about how their bodies work, and the techniques used to mimic the human diseases are well developed. Adult rats and mice will be used for the majority of the work outlined in this project licence as we want the biology of the animals to be fully developed to better represent the patients we aim to treat.

In order to study potential treatments for patients with bleeding disorders, we commonly use animals that genetically mimic the disease symptoms that patients have; for example, in order to study haemophilia A, mice that experience spontaneous bleeding events in the same way that a patient would is crucial for this. Sometimes these symptoms of disease can be severe, with a chance of internal bleeding.

Sometimes, an aspect of a human bleeding disorder is shared more closely with rabbits, for example a protein for clotting that might be present in rabbits but not in rats or mice. Also, larger blood volumes may be needed that rabbits can provide that smaller rodents cannot. Therefore, where justified adult rabbits may be used for carefully selected studies.

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

Most animals will be part of studies that aim to test whether new drugs can prevent or treat the clinical signs associated with bleeding disorders, such as bleeding in the joints or the formation of blood clots. In the majority of experiments the disease will be caused by changes to genes in the rats and mice, which cause them to have symptoms that mimic the human diseases. For example, rats and mice that do not express Factor XIII mimic the disease haemophilia A; like patients these animals can have spontaneous bleeding episodes and require special care. Sometimes these animals may die through bleeding events; although most animals can be humanely killed beforehand, sometimes it is unavoidable for some kinds of bleeding events such as significant internal bleeds. Similarly, handling the animals in order to perform the experiments can also trigger bleeding events. Taken together this is why severe severity is needed for some of the

animals that mimic the symptoms of human bleeding disorders.

As part of the study, animals will be dosed with drugs over a period of days or weeks. Dosing may take place on a daily basis but this will vary depending upon the drug. Drugs will be most commonly given by the intraperitoneal (i.p., into the body cavity), subcutaneous (s.c., under the skin) and oral (by mouth) routes and less frequently by the intravenous (i.v., into a vein) route. For i.p., s.c. and oral routes, conscious animals will be held securely by a trained researcher and the dose administered. For i.v. dosing, animals will be placed briefly in a specially designed rodent restrainer. The animal may be placed into a purpose-built warming cabinet for up to 10 minutes prior to restraint. For rabbits, specific restraint methods will be used; either the most suitable and refined manual method by researchers, or a specially designed device, whichever is most appropriate for the procedure.

Blood samples may be collected during some studies to measure levels of the drug or to assess how well the drug is working. Blood samples are usually small in volume and are most often taken from a vein in the tail, but sometimes from a vein in the leg (saphenous) or, in rabbits, the ear. Sometimes, tests will look at how the animal moves, such as balance and gait analysis (how the animal walks).

The majority of the data will be collected at the end of studies, where animals will be under deep terminal anaesthesia, in order to perform tests on how effective a test agent is at stopping bleeding, or whether it helps form a blood clot. Here, animals are asleep, unaware of any pain, and do not regain consciousness. The animals will then be humanely killed while still under anaesthesia.

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

Mice and rats with a genetic bleeding disorder, such as those that have clotting Factor VIII or IX missing, are models of human haemophilia A or B. As in humans, these animals are likely to experience spontaneous bleeding episodes that may be painful, and these animals are not expected to live as long as normal rodents.

In many cases research is carried out in animals that are anaesthetised, and from which they won’t recover. This means that the animal does not experience any pain or suffering from the procedures carried out, only from the process of being anaesthetised. Under these conditions, we measure blood loss, and the time to stop bleeding from a cut surface (the tail in rats and mice, or the ear vein in rabbits). This mimics that seen in humans where cuts are made in the skin, and how quickly bleeding stops is timed. To induce blood clots we expose blood vessels in anaesthetised animals and cause damage to a vein or artery by adding a chemical or disturbing it with a focused laser beam. The resultant clot can then be either be looked at under a microscope and measured, or it can be removed and weighed. We can measure the time it takes for a clot to block the blood flow through a vessel.

Giving a drug before injury can test its effect on bleeding and clot formation. In some cases drugs are given to the animal whilst it is awake and small blood samples taken to measure levels of drug in the blood, but these procedures are not expected to cause anything other than minimal pain or distress.

In some of the tests, such as when joint bleeds are created, we need to assess how much

pain the animal is in, to see if the potential drug is making a difference. This means that some pain may be experienced by the animal (possibly over a few 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)?

Mice:
Non-recovery - 25%
Mild - 67.3%
Moderate - 3.8%
Severe - 3.9% Rats:
Non-recovery - 57.1%
Mild - 40.9%
Moderate - 2% Rabbits:
Non-recovery - 16.1%
Mild - 79.9%
Moderate - 4%

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 30 January 2029

The PPL holder will be required to disclose:

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

Replacement

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

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

In order to understand the effects of potential drugs in treating disease, the whole "system" must be studied. Animals enable us to mimic the whole biological system, allowing us to study how the vascular (blood-carrying) system interacts with other cells and organs. It is not possible to fully study this in isolated cells and/or organs.

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

Our company regularly uses a range of in vitro (in the test-tube) methods utilising cells to understand how a novel test agent might affect the cellular functioning of those cells. We can sometimes use cells from patients that might help to understand how effective the drugs might be. From these experiments we can prioritise test agents and only take

forward those that have the desired effect and, therefore, look the most promising for the treatment of bleeding disorders. We also use computer modelling “in silico” methods to see how drugs might interact with targets in the body. However, this does not fully answer the research question and studying the whole animal is still necessary, due to how the different body systems work with each other.

Why were they not suitable?

None of the alternatives mentioned can replicate the complete model of the human vascular system, which is required to accurately evaluate the impact of a test agent on a bleeding disorder. In addition, cell-based work does not test the effects the body might have on a test agent, for example how it gets into the blood, travels around the body and how it is removed from the body. No alternative is currently available that can replace the need to test potential therapeutics in a live animal.

A retrospective assessment of replacement will be due by 30 January 2029

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 analysed the number of animals used previously on projects and typically how many animals it takes to fulfil each kind of study, using the most up-to-date experimental methods. This was then combined with a prediction of likely demand of future projects over the lifespan of the licence; this is based on existing relationships with clients, current interest from business development, and the historical likelihood of programme types to make it to the in vivo stage (i.e. pass the stop/go stages from preceding non-animal studies). We have had at least two client programmes concurrently for the length of the preceding licence, so studies are generally ongoing.

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

We have extensive experience in the design of experiments of the types in this project, which has given us confidence in the number of animals required to ensure that no animals are used unnecessarily, but also that the data generated is robust and reliable. We regularly refer to the PREPARE guidelines (https://norecopa.no/PREPARE) and make use of the NC3Rs Experimental Design Assistant (https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction) to ensure that we are using the correct number of animals for every study. We also draw upon any formal training we have had, such as courses from FELASA.

When designing experiments to look at the effect of novel test agents, where the test agent has not previously been dosed before, we look at published literature or client data to determine the variability observed with similar test agents. We can then use the NC3Rs Experimental Design Assistant, or our own in-house developed tool to help determine the most appropriate group size. In addition we have several contacts to draw upon for advice, for example former colleagues.

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

If a new agent is to be tested or refined, small initial experiments (pilot studies) may be conducted first to make key adjustments before proceeding with the larger experiments. This ensures that the correct number of animals are used when experiments are performed in full.

Data from pilot studies and previous experience are used to ensure that the numbers used are as low as possible, without compromising the reliability of the data. Within our company, a member of the wider team has generated a tool for performing power calculations and can be consulted as necessary to assist with study design. This is regularly compared and checked against similar peer-reviewed versions and adjusted where necessary.

Wherever possible, our in vivo (animal) scientists will be blinded to the treatment status of an animal, therefore reducing bias. This enables more reliable information to be gathered from a smaller number of animals. Those who carry out analysis on samples (e.g. blood or tissues) collected during the study are also blind to the treatment status of the animal where possible.

Baseline data (e.g. bodyweight) are recorded and animals are randomly assigned to treatment groups so there is no difference between the groups at the start of the study.

Good planning ensures that within any series of studies we can control for variability that might be introduced by external factors. To limit this variability we use animals of a similar age/weight range, test batches of test agent in the lab first, use the same source of animals and reagents, keep records of all observations made and standardise as many components of an in vivo study as is practicable.

Where possible, we will coordinate with other groups to share tissue including post-mortem tissues to reduce overall animal numbers.

Where genetically altered animals are required, these will usually be provided by our internal breeding projects, which will ensure that animals are bred efficiently using as few animals as possible by communicating need across projects with colony managers. For this we plan out breeding strategies using a projection similar to the Jackson Laboratory breeding colony worksheet. Where animals are obtained from external sources, only the number of animals required for the study will be purchased or imported.

A retrospective assessment of reduction will be due by 30 January 2029

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.

Due to the nature of bleeding disorders and in order to study them, commonly animals that show the same symptoms must be used. For example, rats and mice that have the same (or similar) gene mutations to haemophilia patients are used (missing crucial clotting factors). These animals bleed more easily when injured and can also have spontaneous bleeding events. It is important to use these animals, but there are ways to manage them that reduces overall harm while they are in studies:

Haem A, Haem B, and Glanzmann mice that are used to study bleeding disorders need special daily checks that monitor for bleeding events; this can pick up any bleeds that can be treated with a powder (styptic) if it is on the skin, or that need further monitoring. They also require a change from the usual bedding to a softer variety and the removal of certain enrichment due to their activity levels and propensity to bleed when knocked.

Haem A rats require monitoring over and above regular colony checks; rats vulnerable to having bleeds (homozygous) are given a thorough health check, checking all over their bodies for signs of bleeds under the skin. Signs of bleeds within the body (internal bleeds) are also observed, by for example pallor (very pale extremities like the ears). Their body weight is checked and recorded if there are any signs of ill health. The daily checks pick up bleeds early on, meaning that appropriate action can be taken very soon in the progression of a bleed. Rats with a bleed can be given a treatment to help the blood clot (Factor VIII or Factor VIIa), and any suffering can be eased with pain relief given in Nutella (this means the rats will readily eat it from a syringe tip). Generally, the first treatment and any pain relief reduces any suffering to a minimum and the bleed disappears over a day or two. Animals will be housed in social groups where possible and provided with an enriched environment in order to minimise stress. Animals will be monitored regularly for signs of adverse effects including unexpected bleeding episodes, body weight, activity, responsiveness, condition of coat and posture.

When studying bleeding in joints in mice, we briefly pierce the knee with a very small needle while they are asleep. This method used is very effective at triggering bleeding, while not needing intrusive surgery to change the joint.

Sometimes rabbits are used for bleeding disorder studies. This is because they might have similarities with humans that rodents don’t (how some clotting factors work), or that they can provide a lot more data that a mouse or rat can (for example blood collection over several sessions that would cause problems to smaller animals).

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

Adult rodents and rabbits are the lowest species of mammal that allow us to adequately study the complexities of human bleeding disorders. Although terminal anaesthesia is commonly used for bleeding disorder models, some studies can often take weeks, based on how a disease progresses, or if preventative treatments are being tested. Therefore, although we estimate that a third of our studies will be under terminal anaesthesia, this is not always possible. It is also important that we are able to monitor the behaviour of the animals in a conscious state where relevant. This allows us to monitor for adverse reactions to any new test agents administered and also how the diseases (particularly for joint bleeds) affect behaviour and movement.

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

Animals will be housed in a purpose-built, modern and well-equipped facility that is free of disease- causing organisms such as bacteria, viruses and parasites. They will have access to food, water and where possible items that enhance their environment, such as tunnels, chew sticks and two-storey levels to climb on. Our company staff and the animal care staff are competent in rodent and rabbit welfare and will ensure that animal suffering is minimised. We aim to house animals in groups to promote normal behaviour. However, aggressive behaviour can occasionally result in animals being singly housed to prevent injury.

Each project has a dedicated project manager and a team of highly experienced researchers. This enables us to combine years of knowledge and experience and tailor strategies to refine experimental design as well as the procedures themselves in order to minimise harms to the animals. Open and regular communications with other managers throughout the Establishment alongside unit technicians, Named Animal Care and Welfare Officers (NACWOs) and Named Veterinary Surgeons (NVS) further enables relevant and specific care for our studies and to identify any new and better methods that could be utilised.

To minimise distress in joint bleed mice we will follow measures that include the provision of soft sawdust litter to reduce any irritation on walking, the use of non-tangling nesting material and long nozzles on drinking bottles if movement is impaired. Nutri and hydrogel will also be provided if needed.

All procedures are performed using the smallest needle possible. The lowest volume of blood needed for experiments is determined prior to the study starting to ensure the smallest amount of blood is taken as possible from animals during blood sampling. Injection sites will be monitored for signs of redness, swelling and infection.

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

We use PREPARE guidelines for the planning of animal experiments; these complement the latest version (2020) of the ARRIVE guidelines that are a checklist of important information to include when reporting animal research. Taken together these ensure that animal studies are reproducible, and as translatable to human diseases as possible.

We will also consult Laboratory Animal Science Association (LASA) publications for more

general topical advice.

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

When designing animal studies we consider the appropriate guidelines, including the guidance from The National Centre for the 3Rs (NC3Rs), Laboratory Animal Science Association (LASA), and the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines. This guidance will influence our study design. Searches for publications will also be performed on resources such as norecopa.no to check that relevant 3Rs information is found.

A retrospective assessment of refinement will be due by 30 January 2029

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. Cardiovascular, respiratory and related pharmacology of pharmaceuticals

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Cardiovascular, Respiratory, Pharmacology, Rodents, Large animals

Animal types	Life stages
Pigs	adult
Minipigs	adult
Beagles	adult
Mice	adult
Rats	adult
Guinea pigs	adult
Hamsters (Syrian) (Mesocricetus auratus)	adult
Hamsters (Chinese) (Cricetulus griseus)	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?

This project licence will enable the delivery of cardiovascular, respiratory and related pharmacology (renal and haematological) safety and efficacy studies in both small animal (mouse, rat and , guinea- pig), and large animals (pig, minipig and dog).

A lot of the work performed under this licence will be work mandated by global regulatory authorities prior to first administration to humans or animals in clinical trials. Some work will be non-regulatory, however, such as candidate selection, and pre regulatory testing.

A retrospective assessment of these aims will be due by 08 February 2029

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 we are exposed to are safe or that their potential hazards are well understood and documented.

The data generated from the studies performed under this project will be used to inform decision- making processes on substances under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain clinical trial approval, marketing authorisation or product registration.

This safety assessment is of immense importance along with other non-rodent and non-animal studies in demonstrating to governments and the public the safety of these substances.

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

The principal benefit of the project is the provision of data to facilitate sound decisions on safe/effective product development and appropriate regulatory decisions on clinical trial approval or marketing authorisation for new medicines to which humans or domestic animals will be exposed, thus contributing to their protection and safety.

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

Human and animal patients will benefit from these studies as this work will contribute to the development of new drugs that help alleviate human and animal conditions. 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 devices, and that knowledge may be used to develop further new drugs/devices.

One of the key benefits is the production of data that is required by regulatory authorities, to ensure medicines can be dosed safely to humans or animals. The drugs that will be

tested are for a variety of conditions, in some cases where there is an unmet clinical need to treat such conditions.

In addition, the models on this project may be used to assess the safety or other in life properties of a new drug, and find a dose that causes no adverse effect. This is important when planning future trials in humans or animals, to make sure any starting dose in a clinical trial is safe for the patients taking it.

Our customers will also benefit, as the data we generate will allow them to progress their new drugs into clinical trial, or otherwise if they are found to have adverse side effects.

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 regulatory purposes or to support regulatory purposes (e.g. 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 post- registration 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 safety of pharmaceuticals and devices that can be administered to humans and animals, (either by informing on safety and allowing to progress to clinical trials or preventing pharmaceuticals/devices reaching the market due to 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

- Pigs: 125
- Minipigs: 700
- Beagles: 700
- Mice: 7500
- Rats: 11500
- Guinea pigs: 7500

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 use rats and mice because they are species with the lowest awareness that allow us to get a meaningful comparison as to what may happen in the heart, blood vessels, lungs,

kidneys and blood in humans and other animals. Sometimes though, on specific occasions, rats or mice don't have the physiology to test the safety or efficacy of drugs in the heart (for example). So we have to use other species like guinea pigs, , dogs minipigs or pigs, because they do have the similar physiology to man.

It is a legal requirement in the UK that dogs may only be used in a programme of work involving regulated procedures when the objectives of the work cannot be achieved by using another species. In this project, the dog will only be used when use of the pig or minipig would not achieve the aims of the experiment, or satisfy regulatory authorities. All requests for studies using dogs are assessed by means of an internal review process; the review panel, including scientists, project licence holders and responsible persons under ASPA, consider the information presented to reach a consensus decision, and will only approve the use of dogs where there is robust justification that the study could not be successfully performed using pigs or minipigs instead.

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

Most of the dosing on these studies will be single dosing at a dose that has already been examined in other tests examining different physiology. This information will then be used to fix does in the studies we carry out. Most animals will experience no more than slight effects from dosing and other study procedures. To give an idea, blood sampling procedures and effects would be similar to what a patient would receive when giving a blood sample at the doctors, or having an injection there too.

Animals are dosed by the intended/likely route of human or animal exposure (for example oral administration, injection, infusion or inhalation), and observed regularly to monitor appearance, behaviour and clinical health.

Most animals will suffer nothing more than a scratch from a needle or a little bit of discomfort from dosing or other procedures. Occasionally though, there will be other slightly more pronounced effects (heart rate getting faster, blood pressure lowering) or other effects like weight loss (maybe 10 to 15% from their starting weight), ruffled fur, or bruising at the site of blood sampling. If this does happen then the animals will be carefully monitored, with vets on hand to administer advice and treatments if required. In fact throughout, all the animals are checked at least twice a day by our staff, and any issues or concerns about their health and well-being are reported to more senior staff and or vets who will then examine them and decide what needs to be done next.

Other procedures may be performed to help us carry out our experiments. These include confining animals in special cages to collect their urine and faeces, or in special chambers we use to measure respiratory function, restraining animals during dosing when we are dosing into the lungs (in tubes for rats or mice) and by mask in dogs and pigs, and when fitting jackets to measure heart rhythms. And discomfort will be transient and the animals will be watched or checked regularly during these procedures. If we need to confine animals in special chambers or cages, we often let them familiarise themselves beforehand (habituate) so they are accustomed to their new surroundings.

Some animals may be operated on to implant devices in their abdomen which allow on line recording of blood pressure, heart rate and the rhythms of the heart or to allow the placement of a deep vein catheter for intravenous infusion. Sometimes we actually implant a catheter in the heart to measure pressure inside the heart chambers, for example. The animals will be operated on by vets or other highly trained staff, under sterile conditions in

an operating theatre, and be treated like a human patient would having an operation under general anaesthesia. The animals will be given pain killers before and after, with antibiotics given too under the supervision of a vet. The animals will be allowed to fully recover from surgery before used in experiments. If a vet doesn't think they are fit to undergo experiments they won't be used until they are. These animals may be used again (see above for details) under a very strict criteria as defined by the government, and that will reduce the overall number of animals we have to use in these experiments.

The rest of the animals on this project will be humanely killed at the end of studies. Their tissues and other organs and bodily fluids may be used for other investigations after they are dead.

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 degree of pain or discomfort an animal feels is similar to what a patient would feel having an injection done or blood taken by a doctor.

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 and administer drugs as necessary.

Dosing with drugs may cause adverse effects in some studies, but this is rare as we often have a good idea of doses from other studies. Experience from the last licence shows that the majority (~95%) of animals display only mild severity with the remaining 5% displaying moderate severity. A lot of these moderate severities are due to surgical procedures. Lethality and/or severe effects are not expected to occur, in any of the protocols in this licence.

We 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 consult vets and other senior animal care staff for advice and guidance in its care.

Most animals are expected to experience no, or only mild, adverse effects during the course of the study such as slight weight loss or piloerection (ruffled fur). A small percentage of animals may show more significant adverse effects, such as more marked weight loss, reduced activity, vomiting or tremors. No animals would be expected to die or to suffer prolonged adverse effects as a result of the procedures, and where necessary early humane end-points are applied, under veterinary guidance as necessary, to prevent this; such end-points might include interventions to discontinue dosing, or to provide supportive treatments, or if necessary to humanely kill the animal.

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, about 95% of animals were classified as having experienced mild severity, the rest were classified as moderate. There were no animals classified as Severe at the time of writing.

The moderate severities in the last project would have been largely due to a surgical procedure e.g. cannulation, or an implantation of a device was involved. It's impossible to predict the proportion of severities expected on a service licence like this, as this will be dependent on what study types we are asked to perform. However, a distribution between 'mild' and 'moderate' severities similar to those in the last project are anticipated.

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

- Killed
- Kept alive
- Used in other projects
- Rehomed

A retrospective assessment of these predicted harms will be due by 08 February 2029

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 intact Cardiovascular, Respiratory and Renal systems (heart, blood ,lung, kidney systems) are complex systems which interact with each other in ways that are not fully understood and therefore, there are no adequate models that can be set up in for example a laboratory dish to replace the whole animal experimental model, as the complex mechanisms under investigation cannot be adequately modelled in without the use of whole, intact and functioning animals.

However work in test tubes with cells will be used to help 'screen' suitability of potential substances for further testing in animals. This is because if for example toxicity or signs of abnormal cell development are identified in lab then the test drug/substance will not further progress into animal work.

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

One of the key aims in this project is to look for potential drug induced changes to the heart rhythm. This is a legal requirement for safety reasons, prior to a pharmaceutical being tested in humans.

There are ways we can look at how drugs interact with specific ion channels that influence the heart rhythm in isolated cells (in vitro) using a techniques called Electrophysiology. However, sometimes these tests need to be carried out in animals (usually dogs or minipigs) as the tests on cells don't give any information of the other complex factors involved (like how much available drug is in the heart to affect this).

Why were they not suitable?

Using a tiered testing approach these in vitro tests may be suitable, depending on various risk factors (including the effects previous drugs in that chemical class).

Apart from the example above, although there are in vitro tests that can model some parts of how drugs get into our bodies, and how our body deals with them, and can identify undesirable effects, for example, there is no series of in vitro tests that brings all these complex events together, as in the whole (animal or human) organism. It would be impossible to measure respiratory function and Cardiovascular function, for example, without using a whole animal

That is why we need to test new drugs in animals, as they have similar physiology and processes as humans, and that testing gives us a good idea what may happen if they are subsequently used in humans.

A retrospective assessment of replacement will be due by 08 February 2029

The PPL holder will be required to disclose:

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

Reduction

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

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

The numbers we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received in the past. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

The numbers of animals used in each study are in some cases specified in the regulatory guidelines; where not specified, numbers are based on established minimum regulatory expectation, or on scientific estimates of the minimum numbers required to meet study objectives.

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.

For regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international guidelines where these exist. Otherwise reference is made to standard study designs with input from the Department of Statistics, where appropriate, to identify the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. These internal designs are reviewed and updated in line with changing external guidelines and internal refinements that either minimise numbers or reduce severity.

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.

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.

The experiments we perform are only permitted on the condition we use the least number of animals possible to get a meaningful result to assess safety or whether a drug has the desired effect. These numbers are sometimes based on a number set by a regulator, or based on our own experience. We regularly consult with statisticians when new study types are performed. They do a special calculation (power calculation) which takes into account the size of likely effect we will see to help determine the number of animals we use. Although we do use the least number of animals possible, its important to use enough animals to get a meaningful result, otherwise we would end up using more animals overall than we needed to.

Where we can we only use one control group per study (effectively dosed with the drug formulation without the drug in it) which acts as a baseline to compare any effect of the drug itself. We also re-use animals where this does not adversely impact on the welfare of the animal concerned (see the sections above) and this reduces the overall numbers of animals we use too.

Studies are also carefully designed to combine the different aspects needed to evaluate the safety or whether a drug has its effect combined, and again this reduces the overall numbers of animals used.

A retrospective assessment of reduction will be due by 08 February 2029

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 use adult mice, rats, guinea pigs, dogs, pigs and minipigs We only use dogs when pigs, minipigs or the other species are unsuitable for scientific reasons.

Animals may be implanted with devices to allow continuous recording of cardiovascular parameters such as blood pressure, heart rate etc. This is considered to be a refinement as, despite the initial surgery required to implant such devices, the data obtained is obtained from animals that are going about their daily routines in home pen rather than the animal being taken out of its usual environment
for recordings to be taken, which in itself may be stressful and influence readings. Animals can be group housed in most of these studies.

For all surgical procedures pain relief will always be provided. Surgical procedures will be carried out aseptically and to at least the Home Office minimum standards for aseptic surgery, and in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) (LASA is the Laboratory Animal Science Association). Any animals that undergo surgery will get the same standard of care as a patient who needed surgery in hospital.

All animals are monitored closely and any animals showing adverse effects to test materials will be monitored more closely than normal and appropriate interventions taken depending on effects seen, eg reduce dose, stop dosing, humanely kill. Veterinary advice will be taken regarding any treatment actions needed to stop/minimise any adverse effects occurring.

If we have to repeatedly inject animals or 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 place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. If we can take blood samples when an animal is deeply unconscious then we do so.

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

There is a scientific and regulatory requirement for safety/efficacy data in rodent (mice and rats) and non-rodent species such as dogs or pigs to supplement rodent data and enable a complete risk assessment.

We use pigs or minipigs in preference to dogs wherever possible; (a legal requirement in the UK), and dogs are only used where necessary to achieve the study objectives, ie when the pig is unsuitable (for example due to species-specific differences from humans, confounding pharmacology responses, or practical limitations due to anatomy or physiology).

A lot of the body systems being examined here (cardiovascular, respiratory and renal) are sensitive to the effects of anaesthesia, and data on test pharmaceuticals would be confounded in such cases if anaesthesia was used. So it is not practical to use

anaesthesia for many of the studies we perform, especially for safety evaluation

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

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet the standards in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017). Before we start surgery, we agree with a Vet what pain killers or antibiotics the animals need both before and after the surgery. When animals are recovering from surgery, we give them extra heat and monitor them closely until they are fully recovered and showing normal behaviour. We then check them at least twice daily before they go on study.

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 urine collection), then we would habituate animals to this equipment prior to dosing. Most animals habituate well to this equipment, but if they don't (rare) we remove them from the study.

If we have to repeatedly inject animals or 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 place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. If we can take blood samples when an animal is deeply unconscious then we do so. All personnel performing these procedures are trained to a high standard to minimise adverse effects.

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.

Refinements to improve the animals experience include but are not limited to group housing, environmental enrichment, including novel toys and foods for large animals, and shelters for rodents, human interaction, acclimatisation and training to procedures, and calming measures such as stroking/gentle talking are used to help animals have a better experience of restraint. We have dedicated working groups on animal welfare for each species with a permanent brief to identify potential measures to improve animal welfare, and to trial such measures and make recommendations for adoption.

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

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).

ICH M3(R2) Guideline: Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals; June 2009.

ICH Guideline S6 (R1). PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS (2011)

ICH Guideline S7A. SAFETY PHARMACOLOGY STUDIES FOR HUMAN PHARMACEUTICALS (2000)

ICH Guideline S7B. THE NON-CLINICAL EVALUATION OF THE POTENTIAL FOR DELAYED VENTRICULAR REPOLARIZATION (QT INTERVAL PROLONGATION) BY HUMAN PHARMACEUTICALS (2005)

ICH E14/S7B Implementation Working Group: Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential Questions and Answers (2022)

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

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).
Prior et al. Social housing of non-rodents during cardiovascular recordings in safety pharmacology and toxicology studies. Journal of Pharmacological and Toxicological Methods 81:75-87 (2016).

Sarazan et al. Left ventricular pressure, contractility and dP/dtmax in nonclinical drug safety assessment studies. Journal of Pharmacological and Toxicological Methods 66:71-78 (2012).

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 08 February 2029

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. CNS Neuron-glia interactions, myelin plasticity, and regeneration, in health and disease

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Myelin, Regeneration, Glia, Stem cells, Brain circuits

Animal types	Life stages
Mice	adult, pregnant, embryo, neonate, juvenile, aged
Rats	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 understand how the brain develops and repairs itself in health and disease. We study how stem cells in the brain can become cells that make myelin (a fatty membrane that insulates neurons) throughout life. We want to know the importance of correctly made myelin for brain function, in health and disease. We are looking at how different cells in the brain communicate between each other to control development, and repair, throughout life. The ultimate aim is to learn how to enhance myelin repair or make new myelin as a therapy for several brain disorders.

A retrospective assessment of these aims will be due by 10 February 2029

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?

Neurological disorders are conditions that affect the brain or spinal cord, and are one of the largest groups of conditions without many treatments. They have a large social and economic cost and it is important that we find new ways to treat them.

This research will help us learn more about how the brain grows and works, and how it can sometimes fix itself – like spontaneous formation of new myelin in the white matter of the brain. We will learn more about the pathways and factors that help the brain repair itself. This could lead to new treatments for neurological disorders, such as:

developmental brain disorders; like cerebral palsy, where there is damage to the brain around the time of birth (~17 million affected in the world) and the genetic disorders that are collectively called leukodystrophies, where the white matter doesn't form properly or isn't maintained properly leading to severe disability and death;

acquired disorders such as schizophrenia and multiple sclerosis (~2.5 million affected in the world); and

age-related disorders such as dementia (~50 million worldwide; inc. Alzheimer's disease, frontotemporal dementia and vascular dementia).

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

The aim of this project is to understand how, throughout our lives, stem cells in the brain turn into cells that make myelin, and in disease replace myelin when it is lost or damaged. In disease it becomes clear that myelin is essential for normal brain function, as when damaged or lost it can cause both mental and physical disability.

The fact that myelin can be regenerated provides an exciting therapeutic target for a wide variety of neurological conditions. In fact, the MS (Multiple Sclerosis) society has set this as one of the main therapeutic targets for the next generation of combined treatments along with disease modifying drugs.

The outputs of this licence will likely result in a new information and publications of the following:

Better understanding of how myelin is formed during development, and an insight on how dysregulated developmental myelination can alter brain function. This is important for understanding some neurodevelopmental disorders, and conditions, like cerebral palsy, autism and leukodystrophies.

Understanding on how myelin changes during our lifespan, whether myelination is

regulated differently at different ages, and potentially explain why myelination stops with old age – and why myelin is lost with age. Researching these lifelong myelin changes using approaches that focus on brain function will give us clarity on how myelin influences learning and memory, behaviour and potentially dementia.

One of the main aims of this project is to understand how to promote myelin regeneration, to aid development of new therapies for several white matter disorders like multiple sclerosis. We expect to uncover a pathway to promote myelin regeneration, and test its therapeutic potential.

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

Our ultimate aim is to take the fundamental knowledge we gain in this project and use it to influence potential new therapies that will directly benefit people. This is a very challenging, but the first step is identifying the processes that underlie myelin regeneration and understand how myelin influences brain function. With this knowledge gained we can identify new testable pathways for augmenting regeneration. Some of these pathways may be targeted by using repurposed drugs – facilitating their path to clinical trials.

People with MS, in particular, will benefit from this research by the potential provision of cheap and safe drugs to promote remyelination and prevent progressive disability.

The myelin research field will also benefit from this work as we will advance the knowledge within the field.

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

In order to maximise the output of this project, we plan to undertake the following measures:

We will share all our experiments and findings (both successful and unsuccessful) to the scientific community through publications, conference presentations, and workshops. We aim to upload our results on open access platforms, either before or simultaneously with their publication, so that they can be promptly available to other scientists and to prevent unnecessary duplication of effort.

We will make the results of our project is accessible to the public, enabling those interested to learn more about this field. We have established platforms to actively engage with the public.

We will collaborate with other scientists and/or research and development companies, sharing our knowledge, skills, and techniques to facilitate scientific discoveries.

Species and numbers of animals expected to be used

- Mice: 19000
- Rats: 4000

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.

Myelination only occurs in vertebrates (animals with a backbone) and so non-vertebrate species are inappropriate for understanding myelination and myelin regeneration/repair. There are no myelin regenerative models that have been developed in birds, reptiles, amphibians, or fish. Thus, we use rats and mice because they are the least aware species that can model interaction between brain cells (neurons and glia cells), myelination, cognition (thinking) and behaviour in neurological disorders. The brain circuitry implicated in many neurological disorders is very similar between rodents and humans.

As we are interested in myelin throughout the lifespan we will be using all ages of rats and mice, including older animals so that we can investigate the role of ageing in repair of myelin problems. We are also trialling the use of new-born animals (from birth to 5 days old) for some of our brain injection surgeries as this procedure is less invasive (no drilling of the skull) and requires fewer injection sites in order to get wide coverage of the brain. Another advantage of using the very young animals is that the brain structures needed for the sensation of pain are not yet fully developed before 5 days of age, reducing the overall severity of the discomfort that they will feel.

These experiments will largely be performed on mice, which are essential to this project, as it centres on using the power of genetic modification to highlight mechanisms of how neuronal activity can regulate myelination. However, a substantial proportion of the experiments suggested will be conducted using rats as, due their size, rats are the ideal animal model for toxin-induced myelin damage, in addition to conventional cell culture methods being well established for rat tissue. In some cases genetically altered rats will also be needed for experiments, these modifications will also be used to highlight mechanisms of myelination in experiments which require the increased size of rats and are currently not possible to perform in mice.

There are no demyelination/remyelination models that have been developed in birds, reptiles, amphibians or fish. An overwhelming majority of studies on the biology of CNS regeneration have been undertaken using laboratory rodents, especially mice and rats. Thus, for this work to contribute to mainstream translation-relevant research it is necessary to use mice and rats. These are also the mammals of the lowest neurophysiological sensitivity likely to produce satisfactory results. Much of the proposed work will use tissues after the death of the animal and/or laboratory based methods, however due to the genetic manipulations of the animals this tissue is not generally available from established tissue banks.

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

A variety of different procedures will be performed as part of this license, in most cases animals will recover quickly and without incident (mild severity) or occasionally will be non-recovery, where animals are anaesthetised for the procedure and are killed under anaesthesia. Procedures that fall under the mild category include; injections, drug administration in food or water, blood sampling, breeding and maintenance of genetically altered animals where the alteration is not harmful, behavioural tests, or labelling (with dyes or fluorescent markers) the central nervous system under non recovery anaesthesia. We use drugs or dyes, either injected or fed to the animals, for several different reasons; to activate genetic modifications, to provide pain relief before or after procedures, to label certain cells so that we can identify them, or to change the responses of certain cells to different stimuli e.g. so that we can boost or inhibit myelin formation or maintenance. Once

procedures are completed animals will be humanely killed, the methods used to do this will vary but will be carefully considered to balance the experience of the animal with the usefulness of the tissues that we can obtain after death.

Animals may be aged before or after any of the procedures, they are housed normally and kept until they are the age required for the experiment, this may vary from a few weeks old up to 2 years old.

Injections into the eye are used to investigate how myelin is normally made and ways in which it might go wrong. This is a relatively quick procedure performed under anaesthesia. Repeated eye injections are not typically performed and the total number is limited to two injections.

We use neural implants to either shine light into the brain to activate certain cells, to take pictures of the brain, to record brain activity, or to have a slow release of drugs into the brain over a long period of time so that we don't have to do lots of repeated injections into the brain. We use injections into the brain for 3 main purposes; to change the activity of the brain cells, to label specific cells, or to generate a small demyelinated injury (or lesion, like in multiple sclerosis).

The most severe work involves causing damage to the brain so that we can see how it heals, damage is initiated by injecting a chemical into the area of the brain that we want to have the injury. We have three protocols which involve causing damage to the brain. These protocols are split into the number of brain surgeries that the animal will undergo. Our main protocol involves one surgery but can involve several procedures occurring within the same surgery. Some animals will undergo brain surgery on more than one occasion (maximum of three occasions). In these cases, one surgery will be an injection into the brain, another will be implantation of either a recording device or a cannula attached to a minipump to deliver substances to either prevent or improve repair, and the final surgery would be the injection to generate an area of damage. When animals have multiple major surgeries, they have time to heal and recover before the next surgery, and if they respond poorly and have the most severe side effects then they are not used for subsequent surgeries. Before or after these surgeries animals may receive substances to control the repair, either by injection, infusion, or in their food or drinking water. We may also use the implanted devices to record brain activity or image labelled cells in the brain so that we can see more of the repair process and what cells are responsible for it. We can also use behavioural tests to see how these procedures affect the learning, movement, and capabilities of the animal. Some of these behavioural tests need to keep animals singly housed so that the right animal is recorded (e.g. a running wheel in their cage), some of the behavioural tests need the animals to be slightly hungry to perform the learning task, so they will be food restricted – they do still get fed everyday, just a smaller amount, as this ensures they will like their treats they receive as award for correct answer on a behavioural task. At the end of the experiment the animals are killed by various methods, such as fracturing the neck, perfusion fixation under anaesthesia, or decapitation. Where decapitation is used this is usually in alive and alert adults using a guillotine to ensure a simultaneous separation of the head.

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

Some of the work under this license will lead to moderate adverse effects, these include; genetic alterations which can lead to tremors, maintaining animals as they age so that they

show signs of ageing, eye injections (under anaesthesia) which could cause irritation, brain injections (under anaesthesia) and neural implants (under anaesthesia) which can lead to temporary functional difficulties such as low level problems with walking or head tilts.

For lesioned animals, in most cases the worst clinical sign is a head tilt and some difficulties in balance when moving around that resolves in 72 hours or less. In less than 10% of cases this damage to the brain can lead to spontaneous rolling of the animal or rolling when stressed, or the animal being temporarily unable to right itself. Animals that continuously roll will be humanely killed immediately, and those that cannot right themselves and show consistent reactive rolling will be killed if they do not improve within 8 hours. In up to 15% of animals injected with one of the substances, animals may show a side effect in which they uncontrollably extend their limbs, as this can stop them from being able to move around freely they will be killed as soon as this is seen, this is like a condition called dystonia which is seen in some diseases in humans.

Painkillers will be provided to animals so that they are not in pain from any of these procedures, and during the recovery period they will be kept warm and in a low stress environment, we will also help them to eat and drink if they need it by giving them easily accessible soft food and long nozzled water bottles. Animals will be closely monitored at all times and will be checked on with increased frequency during the recovery period. Surgical procedures will be performed under general anaesthesia and sometimes multiple separate general anaesthesias will need to be used, typically after anaesthesia animals are somewhat disorientated but will be receiving painkillers to minimise any post-operative pain. Animals will only undergo multiple general anaesthesias if there is a scientific need for additional surgery, for example, in one surgery we may need to inject a substance that makes certain types of cells in the area respond to light, allow the animals to recover and the substance time to work, then perform a second surgery in which we make an injury then shine light onto the cells to see if that leads to faster healing. Animals will only be used for multiple surgical procedures if they recovered quickly and completely from the first procedure. In general recovery from anaesthesia should be quick and animals can return to normal behaviour without much intervention. The cumulative effect of any procedures will be considered and kept to the minimum required to yield statistically meaningful data. All animals used in this license are cared for by dedicated staff who follow detailed instructions regarding their care. Humane endpoints have been set throughout this license to minimise suffering, ultimately all animals will be humanely killed and their tissues harvested for further analysis.

Procedures are continually reviewed and refined whenever possible to improve the overall welfare of 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)?

	Non recovery	Mild	Moderate	Severe
Mouse	1%	86%	12%	1%
Rat	6%	44%	43%	6%

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 10 February 2029

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 use of animals is necessary for the current project which addresses several major outstanding questions about the role of myelin in the brain and how to regenerate myelin (the fatty coating around nerve cells) and achieve full functional recovery. The results of this project may have significant clinical relevance to a number of white matter disorders. Existing data show that for most purposes the rodent nervous system is a good model of the human one, both for normal brain function and for the disease processes with which this project is concerned. As the brain is very complex with many different cell types interacting with each other, as well as different environments within the brain, it is not possible to model in a dish (in vitro).

A large part of experiments will be conducted using mice, which are essential to this project, as it centres on using the power of genetic modification to highlight mechanisms of how myelin is formed and its effect on brain function. However, a substantial proportion of the experiments will be conducted using rats as due to their size they are the ideal animal model for toxin-induced lesion.

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

We have considered cell culture based assays; both rodent and human based.

Whenever possible culture experiments, using human central nervous system cells derived from skin cells, will be conducted in specific cell culture petri dishes in the laboratory to reduce animal use. We will also use tissue from dead animals for culture assays to reduce the invasive procedures as much as possible.

Why were they not suitable?

Cell culture based assays do not always represent an appropriate alternative to animal use, thus animal models are needed, especially to study complex environments with different cell types interacting, or to study behaviour, and are essential to understand complex disease mechanisms such as myelin regeneration and changes in the central nervous system with ageing. We know that many brain disorders worsen with ageing and replicating this process is not currently possible in a non animal model.

Though our human cell model is constantly improving we still cannot replicate the complexity of a live brain, such as in learning and memory, or the factors affecting myelin formation, damage, or repair such as in diseases like multiple sclerosis.

Existing data show that for most purposes the rodent nervous system is a good model of the human one, both for normal function and for the disease processes that we are investigating in this project. Thus, making it our best choice to investigate these complex interactions.

We use cell culture models in conjunction with animal models to provide a hierarchy of models from which a full and detailed understanding will emerge. Where possible, if cell culture models will suffice to answer the question, then they will be used in preference to animal work. As cell culture models invariably involve the use of neonatal tissue, they do not fully replicate the effect of adult ageing that are critical to achieving the programmes objectives so cannot replace all the animal work. This holds true for human cell culture models as cells derived from induced pluripotent cells also mimic foetal cells, more than mature brain cells.

A retrospective assessment of replacement will be due by 10 February 2029

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 establishing and maintaining each transgenic rodent line, we will follow available guidance on good breeding practices. We estimate a minimum of 5 cages per line: 2 cages for the breeding pairs, and 2- 3 cages for offspring and breeding stock animals (separated males and females after weaning).

Equalling a total of 10-15 animals per line per week. This is the minimum number that allows for an uninterrupted flow of offspring (taking into account the <100% mating success, and the fact that at any time some of the colony will be below breeding age). To keep breeding and maintenance to a minimum for the duration of the project licence, we will cryopreserve each line and only keep lines active that are being used for experiments. Additionally, we have used our annual return of procedures data from our previous licence to estimate the numbers of animals we will need for breeding.

Experimental groups, and the number of animals required for statistically significant results, will be determined from our previous work, published data from others, and non-animal work used to identify potential mechanisms or pathways of interest. Routes, dosage volumes, frequencies and durations will be obtained from published literature where available. Pilot studies will be used to confirm that the chosen parameters are well tolerated by our specific models and genetic backgrounds, and to provide estimates of the expected variability and the size of the effect so that we can set the group sizes appropriately. Robust control groups are required to ensure validity of data – our surgical models can themselves cause responses not related to the experimental question being tested, but as a consequence of damage to the central nervous system from the implant or injection. In experiments where we implant a device for monitoring animals over multiple timepoints, we will use the initial recordings from before additional procedures to act as a control.

We will use randomisation tools in order to assign animals to experimental or control groups, and analysis will be performed using computer automation or with experimenters blinded to the conditions.

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

In all experiments we aim to use the minimum number of animals compatible with obtaining scientifically and statistically significant results. Experimental groups are chosen to contain age, sex and strain matched animals as far as is possible (on occasions where, for example, poorly breeding transgenic lines are used then groups may have mixed sexes in equal numbers).

To minimise variability control animals will be obtained at the same time and from the same supplier (or littermates if bred in house) as the animals under experimental protocols where possible, housed in the same conditions, and procedures performed by either the same researcher or with conditions divided evenly between researchers. Within the same experiment the same batch of drugs or reagents will be used for all groups.

Wherever possible data will be analysed blinded to experimental treatments until after the final statistical analysis.

The animal numbers used in experiments will be based on the number of different manipulations being carried out, and guided by animal usage that restricts animal numbers

to the minimum. Preliminary experiments and existing literature will be used to calculate, using reputable statistical experimental design tools, required sample sizes prior to the any full experiments being carried out, to ensure that we generate statistically meaningful data.

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 further reduce overall animal usage in this licence, where possible we design experiments across experimenters in the laboratory in such a way that tissue from an animal can be used for other experiments, for example one researcher using the brain, and another the optic nerve, or sharing of embryonic tissues so that the full litter can be used. Furthermore, we bank tissue from our transgenic animals for a few years, so these tissues can be used for future experiments instead of needing to set up a new experimental procedure.

Where cross fostering of pups is required, foster mothers will be preferably sourced from within our ongoing breeding colonies rather than having additional matings set up as potential foster mothers.

We have established and will further explore, replacing some cross-sectional studies (where animals are killed at different timepoints) with longitudinal studies (where the same animal can be used to give results at different timepoints), to both reduce animal number but also improve the scientific output of those studies.

Whenever possible culture experiments will be conducted, however, cell culture based assays do not always represent an appropriate alternative to animal experiments. Thus, animal models are needed, especially to study complex environments with different cell types interacting, or to study behaviour. From our preliminary cell culture work using rodent myelinating co-culture, and primary oligodendrocyte cultures we have already identified candidate pathways and genes that may regulate myelination, this preliminary work reduces the numbers of animals used for the animal work as we are only taking forward promising candidates from our preliminary data.

We have access to a recently set up user run email list, where surplus wildtype animals generated from breeding and maintenance protocols can be shared. We have used this list to offer and obtain animals for control tissues, with the aim to minimise the purchase of wildtype animals where possible.

When our GA rats have reached the end of their breeding and maintenance protocol, we have coordinated with other labs to provide them with tissues or blood after sch1 killing. We also do this with our wildtype rats bought in for tissue culture whenever possible.

A retrospective assessment of reduction will be due by 10 February 2029

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.

These experiments will largely be conducted using genetically altered mice. However, a substantial proportion of the experiments suggested will be conducted using rats as, due their size, rats are the ideal animal model for toxin-induced lesion, in addition to cell culture methods being well established for rat tissue. In some cases genetically altered rats will also be needed for experiments, these modifications will also be used to highlight mechanisms of myelination, in experiments which require the increased size of rats and are currently not possible to perform in mice. We use rats and mice because they are the least sentient species that can model glia-neuron interaction, myelination, cognition and behaviour in neurological disorders.

For ageing experiments, animals will only be kept if they are showing no, or minor and controllable, ill effects from growing older. Teeth checks and general health monitoring ensures that animal suffering is kept to a minimum.

To induce gene expression or to modulate or deplete specific cells, some animals will be given substances by mouth, injection, oral gavage, or in their drinking water or food. We also use behavioural tests in some animals to see the functional effect of our modifications. The behavioural tests chosen are mostly observations of movement or teaching animals to use a touch screen and ‘punishments’ are in the form of lights or noises rather than electric shocks or other painful punishments.

Injections into the eye are used because the optic nerve is normally fully myelinated with a well known timeline of myelination and this makes it an ideal model system that allows us to investigate how myelin is normally made and ways in which it might go wrong. Eye injections are safe and are routinely used in human patients. The eye injections take place under general anaesthesia (human patients receiving eye injections generally just have local anaesthesia) to ensure that the distress of the animals is kept to a minimum.

For some experiments we will use injections into the brain or spinal cord, with or without implanting a device to record or stimulate the area, this is the most refined method to provide a local effect on the brain. Targeted manipulations in this way are more refined than disrupting the whole brain or spinal cord. We also use injections to transplant cells into the central nervous system.

In our most severe work, we will use brain lesion models in order to investigate the myelin regenerative (healing) process. Our lesion models create small areas of myelin damage and have been chosen partly because of the minimal behavioural and movement side effects. For example, our primary model does not cause progressively worsening symptoms, i.e. the animals are able to move freely and perform normal physiological functions, like feeding, drinking, grooming and movement after the initial surgical recovery period. Our lesion model is carried out by performing an injection under anaesthesia into a specific area of the central nervous system, animals get better over time and recover back to their pre-lesion state of fitness within 1-3 days, though the symptoms immediately post

lesion surgery can be severe and distressing to the animal. Other lesion models can cause lasting or progressive symptoms such as paralysis or movement problems.

In undertaking these procedures, we are continually assessing how the procedures can be refined in order to minimise the discomfort that the animals may experience and seeking to replace procedures using live animals (in vivo) with procedures using tissues after death (ex vivo) where possible.

We take the welfare of the animals very seriously and routinely use pain relief and provide appropriate supportive care during surgical procedures, we familiarise animals to handling for procedures where handling is necessary, and increase monitoring of animals that have undergone invasive procedures so that we can quickly identify and treat or minimise any adverse effects arising. Stress can also affect the healing process that we are investigating so it is important for the animals to be in the best possible condition not just from a welfare perspective, but also to make sure that we get good data. The humane end points proposed are a balance between the suffering of one animal and the potential need for a replacement animal to undergo procedure. Due to the nature of the processes that we are investigating, some clinical signs associated with myelin deficits are to be expected, we have already set our humane endpoints to be prior to the onset of more severe clinical signs.

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

Myelin only occurs in vertebrates (animals with a backbone) and so non-vertebrate species are inappropriate for myelination and myelin regenerative studies.

We have tried to establish a model to study developmental myelination and its role on brain development in a frog larvae. However, experiments are confined to the early developmental period, and experimental methods to study myelination are not well adaptable to this model making it hard to use for some of the fundamental questions on myelination. Furthermore, it is not suitable as a model to study the role of myelin and glia on neuronal circuit function with age and myelin regeneration.

Indeed, there are no demyelination/remyelination models that have been developed in birds, reptiles, amphibians or fish.

An overwhelming majority of studies on the biology of CNS regeneration have been undertaken using laboratory rodents, especially mice and rats. Thus, for this work to contribute to mainstream translation-relevant research it is necessary to use mice and rats. These are also the mammals lowest on the evolutionary tree likely to produce satisfactory results. Much of the proposed work will use ex vivo tissue and/or in vitro methods, however due to the genetic manipulations of the animals this tissue is not generally available from established tissue banks.

Currently the rodent nervous system is the most suitable model of the human one, both for normal brain function and for the disease processes with which this project is concerned.

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

We have refined many steps in our procedures to minimise welfare cost to animals, and we will continue to do so for the duration of this licence. Wherever possible we will use the

least invasive method of administration and the minimal number of surgical interventions required to generate meaningful data.

Over the course of this license we will seek out potential refinements by;

Evaluation of alternative forms of tamoxifen (a gene inducing agent) to minimise the ill effects associated with its use.

Frequent monitoring of animals during recovery periods.

Personal license holders working under this license will meet regularly to review the methods used and identify any issues or areas of potential improvement.

Attendance at locally run method workshops, such as tamoxifen workshops, where the different methods of administration are discussed in terms of how well they work and animal experience. Where the conclusions of the workshop represent an improvement on current methods without compromising ongoing experiments we will switch to the new method if covered under this PPL and/or amend this license if required.
Monitoring the field for new developments and technologies to improve procedures. For example, we will test the use of powered minipumps with a larger reservoir rather than needing to replace osmotic minipumps.

The refinements we have established under our previous licence or within this new license are as follows:

We have refined our methods to minimise stress to the animals by administration of post-operative pain relief in jelly – this means that the animals often do not need to be restrained in the recovery period post-surgery and do not need analgesia injection (other than the pre- operative analgesia). This refinement has been widely shared and implemented in other labs.

We have further extended our jelly refinement to cover administration of experimental test substances in jelly/chocolate spread wherever possible, thus minimising the need for daily injections or minipump implantations.

We have incorporated the use of minipumps to replace large numbers of injections, to reduce the handling stress to the animals and minimise the risk of needle injuries, to be used when administration in the food or drinking water is not a suitable route for dosing animals.

We have introduced the use of an analgesic cream on the ear bars of the stereotactic frame to minimise post-operative discomfort.

We have refined the grading system from previous licenses based on our increased surgical experience and introduced some additional grades and clarification of how animals will be graded to maximise replicability of severity reporting between users, further detail on the grading system is found within relevant protocols. Animals that are not showing signs of improvement will be killed, but those animals showing consistent improvements over time without reaching the humane end points detailed within the license will be kept such that future surgeries are minimised.

For our most severe protocols involving multiple stereotactic surgeries, we have introduced

limits so that animals experiencing severe side effects after the initial surgery are not used for any subsequent surgeries, to ensure that animals do not experience severe suffering twice.

We have implemented a non-recovery protocol for injection of fast-acting-labelling substances into the brain to minimise the overall severity experienced by the animals and avoiding the adverse effects associated with brain injections and surgery.

We have developed body condition score sheets and/or clear grading systems based on input from the NVS, guidance on actual severity reporting, the adverse effects described in the literature and adverse effects encountered in our own experience. These score sheets and grading systems will be made available to animal technicians as well as personal license holders to ensure that there is reproducible identification of potential problems as well as recognition of subtle combinations of effects which could impact on animal welfare. These score sheets and grading systems will be regularly reviewed to ensure relevance and accuracy.

We have generated a new mouse model an alternative to the shiverer mouse, this has numerous advantages over the shiverer mouse as they are overtly normal and do not show seizures, tremor, or the reduced lifespan that is seen in shiverer mice. This will be shared with the scientific community to minimise the overall use of the shiverer mouse worldwide.

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

We aim to follow the best practice guidance to ensure experiments are performed in the best possible way. To achieve this, we do the following;

We adhere to the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

We have and will continue to adopt the Laboratory Animal Science Association (LASA) Guidance on Preparing for and Undertaking Aseptic Surgery (2017) and the Home Office Minimum Standards of Aseptic Surgery. We participate in workshops relating to surgical procedures and follow the literature on how to keep our methods up to date with the best possible practise.

For breeding we observe the guidelines provided by the Home Office and the NC3Rs Resources on 'Genetically altered mice' and reputable laboratories for best practices to breed genetically altered rodents.

We have refined methods to administer drugs, and chemicals, we always seek the least invasive method, such as minimising injections of painkillers after surgical procedures by administering in jelly or chocolate spread. We will continue to develop new methods, and follow new developments in the field, on refining procedures for the administration of substances. Again, we will refer to the NC3Rs website for guidance.

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

We receive the NC3R newsletter that highlights latest NC3R publications and advances. We will regularly attend available workshops on welfare. We regularly discuss our work with the animal facility staff, and named veterinary surgeons, as they are aware of new developments and have good guidance.

We are active users of our 3Rs search tool, that is regularly updated. This database for example contains helpful information on ways to reduce the harm to animals used in research. In addition, it has helpful advice on how to reduce the number of animals we use in experiments. 3Rs updates and suggestions are regularly shared with license holders.

Within the neuroscience field, when new technologies become available they are often presented at conferences or published in scientific journals, we attend numerous conferences and frequently check available publications for advances in the field. Where these new technologies offer the opportunity for reduction or refinement of animal procedures, in a way not covered by our license, then we will apply for an amendment to allow pilot studies of their use. Where these new advances offer the chance of replacement of animals then we will compare them to our existing animal models to validate them for our research questions prior to adopting the new technology or technique.

A retrospective assessment of refinement will be due by 10 February 2029

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. The diversity of glia in health, development 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

Glial cells, Glial pathologies, Myelin, Gene therapy, Neurons

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?

Our project aims to investigate the various cell types in the brain (such as neurons and glial cells) and how they affect our well-being. We will prioritize understanding how genes impact the function of these cells, and use that knowledge to develop innovative therapies for brain diseases, specifically the rare Pelizaeus-Merzbacher Disease (PMD), which has an incidence rate of 1 in 200,000.

A retrospective assessment of these aims will be due by 28 February 2029

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 long "arms" of nerve cells are wrapped in a layer called myelin, which helps electrical signals travel quickly along the nerve cells. This myelin is made by special cells called oligodendrocytes in the brain and spinal cord. If the myelin breaks down, it can cause nerve cells to die, leading to serious problems like Pelizaeus-Merzbacher disease and multiple sclerosis. We want to learn more about the genes that are involved in making myelin and how they work, so we can develop treatments for these diseases. In this study, we will test potential treatments for Pelizaeus-Merzbacher disease, which currently has no cure.

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

Our research is focused on understanding how glial cells work. We want to learn about the different genes that are important for these cells to make a substance called myelin, which helps brain cells communicate with each other. We also want to understand how glial cells are organized in the brain and how they interact with other brain cells.

We will create a new model to study a specific disease called Pelizaeus-Merzbacher disease, which affects the myelin in the brain. We hope that this will help us develop new treatments for diseases that are caused by problems with myelin.

Our findings will be shared with other scientists by publishing them in peer-reviewed scientific journals.

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

We are creating for the first time a special type of mouse that has the same genetic mutation as humans with Pelizaeus-Merzbacher disease (PMD). This will be very important for scientists because it will help us understand the disease better and develop new treatments.

If the treatment we develop for PMD in this project works well, it could also be tested for other diseases that affect the same substance in the brain, like multiple sclerosis (MS).

In the future, if we find successful treatments in our animal tests, they might be used to treat humans with these diseases.

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

We will be presenting our ongoing research results, including any failed attempts, at scientific conferences like the European glia meeting. We'll also publish our findings on platforms like F1000research, so that other researchers can benefit from our work. By

sharing our knowledge and collaborating with other research teams, we hope to advance the development of gene-editing therapies and promote the spread of new knowledge gained through our project.

Species and numbers of animals expected to be used

- Mice: 4000

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 have been the primary model for studying myelin biology, including the genes necessary for myelin production and brain development, as well as those involved in developmental diseases such as PMD. We plan to study these genes during the developmental stages of mice. Furthermore, we have found that isolating brain cells from mice at an early developmental stage for in vitro studies (using cells in a dish) is more effective and reliable than using adult mice. It's important to note that pre-clinical data obtained from mice is highly relevant for translational medicine, as it helps to inform and guide the development of potential treatments for human diseases.

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

Generation of genetic altered mice: We will use breeding techniques to create a group of animals that have been modified in a particular way, such as turning off certain genes, increasing the expression of other genes, or introducing specific genetic mutations.

Substance administration: To study the effects of certain genes or drugs on mice, we need to introduce them artificially into the mouse's DNA. This is done through a process called genetic engineering, which creates what is called a "Transgene." We will also test drugs treatment that have been validated in a lab setting (in vitro). We will administer these Transgenes or drug candidates to the mice through injections, using different routes depending on the substance being tested. For example, Transgenes might be given to mice through an intravenous injection (into a vein) once, while drug candidates might be administered through an intraperitoneal injection (into the abdomen) daily for a week. We will follow established guidelines for animal welfare and make sure that the doses and frequency of injections are safe and appropriate.

Behavior test: Assessment of cognitive, sensory and motor coordination might be done for mice developing phenotypes. For example, The rotarod behavior test is a commonly used test to evaluate the motor coordination and balance of mice. It involves placing a mouse on a rotating rod or drum, which gradually accelerates. The test measures how long the mouse can stay on the rod before falling off or being removed by the experimenter. During the test, the mouse needs to maintain its balance and walk or run on the rod to avoid falling off. This requires the mouse to use its coordination, balance, and motor skills. The longer the mouse can stay on the rod, the better its motor coordination and balance are considered. The test can be performed using different protocols, such as varying the speed of the rod or the duration of the test. It can also be used to assess the effects of different drugs or genetic manipulations on motor coordination and balance in mice.

Health check: Daily health checks can be done if mice develop harmful phenotypes.
At the end of the experiment all mice will either be humanely killed, or tissues and organs collected under deep, terminal anaesthetic unconsciousness. This process starts by first removing the blood by pumping a salt-containing liquid through the blood vessels, called perfusion, followed by tissue preservation in a fixative solution for follow-up analyses

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

In this license, the majority of animals being used (about 75%) are not expected to develop any significant changes in their physical or behavioral traits that will persist throughout their lives.

However, mice with mutations in their myelin genes may develop issues with their brain and nervous system, which can lead to problems with thinking, sensing their surroundings, and coordinating their movements. These symptoms may appear when the mice are about two weeks old and may persist throughout their lives.

In severe cases, the mice may also experience tremors and seizures by the time they reach three weeks of age, and may start losing weight as a result. These mice will be humanely killed if they develop seizures or if weight loss exceeds 15%.

When mice receive injections of transgenes or drugs, they usually only experience minor changes in their appearance or behavior. These changes are not higher than moderate in intensity. For example, some compounds may cause change in development leading to small pups compared with control or untreated littermates, or injecting Tamoxifen might cause the mice to temporarily lose weight.

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: 25%

Mild: 50%

Moderate: 20%

Severe: 5%

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

- Kept alive
- Killed

A retrospective assessment of these predicted harms will be due by 28 February 2029

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 study of how glial cells are arranged and interact with other cells in the brain requires the use of animals. This is because the brain is too complex to be fully replicated in a lab setting. To learn how glial cells work in the brain and how they communicate with other brain cells, we need to study animals because the brain is very complex. It's impossible to replicate a whole brain in a lab that works the same way as a real one. To do experiments in the lab with brain cells, we have to take them from living animals. We also need to study live mice to see how neurological diseases affect them and if new treatments can help.

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

We considered using cell lines (e.g. HEK cells, Glioma stem cell lines...), stem cells-derived neural cells, cerebral organoids (Cerebral organoids are three-dimensional structures that are grown in the laboratory to resemble certain parts of the brain. They are made by culturing cells that can develop into different types of brain cells, which then self-organize and form structures that resemble the brain's tissue) and in silico modelling (computer models are used to simulate the behavior of molecules or cells).

Why were they not suitable?

We currently do not have any cells lines that can make myelin, which is a substance in the brain that helps cells communicate with each other. Scientists have been trying to use different methods, such as growing special types of cells from stem cells or making mini brain-like structures called organoids, to study how myelin works in the brain. However, these methods are not very good yet and we haven't been able to see myelin being made in the organoids. These models are also not very useful for studying how glial cells are organized in the brain.

A retrospective assessment of replacement will be due by 28 February 2029

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 project involved using mice that had been genetically altered in specific ways. To study one particular gene, most of the mice needed to have an enzyme called "Cre recombinase" present alongside the gene. This meant that each mouse had to be bred with another mouse that had the Cre enzyme, in order to activate the mutation in the gene they were studying. Because of this, we needed to use a larger number of mice to carry out the research.

Some genetically altered (GA) animal used in this project present recessive mutations in the chromosome X (A recessive mutation is a specific change in a gene that only has an effect if both copies of the gene carry the mutation. A recessive mutation located on the X chromosome means that the specific gene change is on one of the sex chromosomes. In males, it can cause phenotypes because they have only one X chromosome. In females, they may be carriers without symptoms).

PMD is a congenital leukodystrophy that presents a recessive mutation in a gene present on the X chromosome. Therefore, only boys develop the disease, and no cases of girls developing PMD have been reported in scientific literature. To ensure the most effective treatment for PMD, we will use male mice with PMD as our primary focus during the development process. This approach aims to make the treatment applicable and relevant to human patients. The females we will produce through these breeding can be used for further breeding to generate more male PMD mice, as they will carry the mutation without developing the disease.

The number of animals used in this project has been reached by carefull consideration and discussion with current PPL-holders for projects similar in character and scope to what we aim for and expect (protocols, number of mouse lines used).

Based on these interations we have performed approximate power calculations in order to gauge a likely number of animals required for completion of the project. Balancing statistical power and reduction of animal use.

To minimize the harm to animals, we will perform experiments using primary brain cells isolated from mice pups in a lab instead of using the whole animals. This means that most of the litters of mice pups will be used for these experiments instead of being raised as live animals.

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

The NC3Rs Experimental Design Assistant (EDA) will be used to plan experiments in a way that uses the fewest possible number of animals while still achieving the scientific goals. Additionally, the EDA provides guidance on how to minimize any subjective bias in the experiment and how to conduct appropriate statistical analyses to ensure accurate results.

PREPARE guidelines will be used for planning animal experiments.

We will follow resources found on the NC3Rs website (e.g., to help us determine the appropriate number of animals to use in our experiments, plan pilot studies, plan breeding and colony management).
Good breeding design will be implemented to minimize the number of unwanted genotypes.

All researchers working under this license will have received basic training in statistics by attending courses. When needed, we will consult specialist statisticians regarding experimental design and statistics.

We will maximize the use of experimental tissues/biological samples that are surplus to the original experiment by making them available to other researchers to address other biological questions that are within the scope of the current project aims.

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

Pilot study will be design for each new GA animals and new protocols involving drug or transgene injection to test the feasibility and practicality of the experimental design before conducting a full-scale study. A small number of animals will be used to test the experimental procedures, refine the experimental design, identify any potential issues that may arise during the experiment and amend the licence if needed. This helps to reduce the risk of errors and increase the reliability of the results obtained from the main study. The data obtained from a pilot study can also be used to estimate the sample size needed for the study and to determine whether the proposed study is likely to achieve its objectives.

Computer modeling, power calculation, and sample size calculation will be used for each experimental design.

Coordinated breeding between each user of this project will be done to minimize the number of breedings and animals used.

Breedings will be optimized to acquire the maximum output.

Mouse and tissue sharing, including tissues from genetically modified mouse lines and post-mortem tissues, will be done to further reduce the overall number of mice used.

Several tissues from the same mice will be used.

Mice will be placed in experimental groups randomly, and blinding will be applied to prevent any potential bias in the results obtained from the experiment. This ensures that the study is conducted in an objective and scientifically rigorous manner.

A retrospective assessment of reduction will be due by 28 February 2029

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 rodents as animal models because we have the most experience and technical expertise with them. Specifically, we will use mice instead of rats because mice are less sensitive to social stressors such as being separated from their mothers during weaning. This will help to ensure that the animals used in the project are as comfortable and stress-free as possible.

Whenever possible, we will use genetic modifications that can be turned on and off (inducible) rather than ones that are always active (constitutive) to prevent any negative effects that are not directly related to the goals of the experiment. In this project, we are investigating the role of certain genes in adult mice. Therefore, we want to avoid causing any developmental problems in the mice, as our focus is on understanding adult mechanisms. This approach is important to prevent unnecessary pain, suffering, and distress to the animals.

We will prioritise non-invasive delivery for drug administration. E.g. Drugs administred in drinking water or jelly. Intraperitoneal injections rather than intracerebroventricular (into the fluid'filled space.s in the brain called ventricles).

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

Mammals have highly developed central nervous systems. Glial cells are a crucial component of this system and have evolved in a unique way in mammals, setting them apart from other commonly used animal models such as worms, fruit flies, zebrafish, and reptiles. We will be studying glial cells to understand the mechanisms that are involved in human diseases related to the nervous system, such as myelin disorders like Pelizaeus-Merzbacher disease (PMD). This research will help us gain a better understanding of these conditions and how to treat them.

There is as of yet no model of PMD in Zebrafish.

The overwhelming majority of studies on myelin biology have been undertaken using laboratory rodents, especially mice.

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

To improve the well-being of mice, all mice will have acces to enrischment and enhancements. This can involve housing them in groups to allow for social interaction, as well as adding materials like nesting materials, chew blocks, and wood shavings to their cages. Other enrichments like tunnels, climbing structures, and new objects can be added

to encourage natural behavior. Finally, offering food in different locations within the cage can encourage foraging behaviors and make the mice feel more at home.

Mice experiencing mild, moderate or severe symptoms (such as reduced motor and sensory function) or living in breeding cages where their pups will be collected, we will provide additional environmental enrichment (as listed above)

For genetically altered animals potentially leading to severe phenotypes, we will establish intensive monitoring programme and clear endpoints (for example, a monitoring programme for experiments expected to cause neurological signs in animals will be created in form of a checklist, which covers general body condition and objective assessments of neurological function such as posture, movements, presence of tremor and seizure).

In this project, we plan to change important genes using a method called inducible gene modification. We will use a common technique called drug-induced cre-lox mediated gene recombination, which involves giving the mice a drug called tamoxifen to activate the gene modification. However, we have noticed that tamoxifen can cause some temporary side effects, like weight loss, because it reduces the mice's appetite. To reduce this impact, we will give the drug at a time when the mice are less likely to be affected, and we may use a flavored jelly instead of oral gavage. If we need to give the drug in their drinking water, we can add sweeteners to make it taste better.

When possible, we will encourage voluntary treatment for experimental treatment as well as pain management using medicated palatable substances such as mediated chow (food that contains medication mixed in with the regular chow), medicated flavoured jelly/paste, medicated sweetened water.

Whenever we are trying out a new procedure that might have obvious negative effects, we will begin with a small test to see how it goes (pilot test). We will also set up a system to keep track of what is happening and try to minimize any harm or discomfort.

When possible, we will do a test on genetically altered mice to see if we can answer our scientific questions by looking at mice that only have one copy of the mutation (heterozygous mice) and show less harmful physical characteristics.

For each mouse line, a detailed phenotypic assessment will be made.

By minimizing the handling of mice that are likely to experience seizures, we aim to reduce the occurrence of seizures.

In order to prevent seizures that can potentially result in the death of the mice, we will carefully evaluate and identify early clinical signs that indicate the likelihood of a seizure occurring in a pilot study. By identifying these early signs, we can intervene sooner and implement appropriate measures to reduce the occurrence of seizures. In humans, early symptoms associated with Pelizaeus- Merzbacher disease include nystagmus (rapid and involuntary eye movement), weakness (lack of strength or power in the muscles), spasticity (difficulty controlling muscles, resulting in stiffness and tightness), stridor (high-pitched sounds when breathing), dysphagia (swallowing difficulties), and walking difficulties. In mice that are likely to experience seizure, pilot study including Eye movement check, Grip strength behaviour test (the animal grasps a grid or another object, and the object and animal are pulled apart until the grip is released), Assessment of

mouse posture (check abnormal hind limb posture and loss of hind limb extension reflex when mice are suspended by the tail), Mouse weight measurement, Evaluation of mouse walking behaviour and Intensity of shivering will be done to identify early clinical sign. During this pilot study, the mice will be checked three times a day. After completing the pilot study, we will refine and adjust the frequency of these check-ups. The goal is to be able to identify these early clinical signs while minimizing the amount of handling the mice undergo

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

We will follow technical updates on animal experimentation and welfare with articles published in "Lab animal" (https://www.nature.com/laban/) especially paper published by the NC3Rs team.
We will follow PREPARE guidelines for planning animal experimentation as well as ARRIVE guidelines when drafting manuscripts that detail animal research.

To make sure our experiments are done as accurately and ethically as possible, we will look to the advice and guidance provided by organizations like the NC3R, the Laboratory Animal Science Association (LASA), and the International Mouse Phenotyping Consortium (IMPC)

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

The NC3R website can provide help and guidance to researchers working under this project to implement the 3Rs in experimental designs (https://nc3rs.org.uk).

We will seek guidance from both the Laboratory Animal Science Association (LASA) and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) for the latest recommendations and advances in animal research techniques. Furthermore, we will use online resources provided by the Jackson Laboratory Resource Library, as well as the latest regulations, policies, guidelines, and databases provided by Norecopa, to implement the 3Rs in our work.

In undertaking procedures in this project, we are continuously assessing how the procedures can be refined in order to minimize the discomfort that the animals may experience. Members of the lab holding personal licenses who undertake procedures under this project license meet regularly to review operational procedures.

We maintain regular communication with animal facility staff in general and NACWOs and NVS in particular.

A retrospective assessment of refinement will be due by 28 February 2029

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. Modelling and treating neurodegeneration

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

Nervous system, Neurodegeneration, Therapy, Neurological diseases, Motor neuron disease

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

Develop a better understanding of how and why the nervous system breaks down across a range of different neurological conditions, ranging from Alzheimer's disease through to motor neuron diseases such as spinal muscular atrophy [SMA] and amyotrophic lateral sclerosis [ALS]. Use this knowledge to design safe and effective treatments to prevent or

delay nervous system breakdown.

A retrospective assessment of these aims will be due by 08 March 2029

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?

Dieases of the nervous system, like Alzheimer's disease and motor neuron disease, are some of the most debilitating a devastating to affect global human populations. Unfortunatley, for the vast majority of these conditions, no effective treatments are currently available. In order to develop treatments for these conditions, we first need to develop a better understanding of how and why the nervous system breaks down. This knowledge can then be used to develop the next generation of effective therapies for human patients.

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

This project will undertake both fundamental biological research, as well as translational/pre-clinical research in order to identify and develop therapeutic targets and strategies for neurodegenerative diseases such as motor neuron disease and Alzheimer's disease. One of the particular strengths of our approach is that we aim to identify targets and therapies that are relevant to more than one neurodegenerative condition, meaning that therapies developed and tested using one disease platform in the short to medium term have the potential to be successfully applied to other, related neurodegenerative conditions in the medium to long term.

The results of the research performed during this project will be disseminated in leading international research journals, through presentations at international research conferences, and through our links with leading global patient charities . Thus, the project will add significantly to the scientific literature and therefore assist other research labs around the world pursuing a range of diverse projects in an attempt to deliver new options for patients with neurodegenerative conditions, whilst also keeping the patient community updated on progress (e.g. through social media channels).

Benefits Summary:

Short Term (1-3 years) = i) Gain new insights into disease mechanisms relevant to other practising neuroscientists as well as drug development laboratories (academic and commercial) seeking new therapeutic targets to develop; ii) Continue to develop and optimise therapeutic strategies for motor neuron disease (e.g. targeting of PGK1 with drugs such as terazosin) that have already made it in to human clinical trials in order to maximise chances of success

Medium Term (3-5 years) = Identify additional novel therapies suitable for moving towards clinical trials in human patients with neurodegenerative conditions relevant to both the scientific and clinical communities (as well as patient interest groups who play an important role in establishing and supporting clinical trials)

Long Term (5-10 years) = successful development and rollout of novel therapeutic strategies for neurodegenerative disease that will directly impact on patients and medical professionals (including the NHS)

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

Short Term (1-5 years) = i) Researchers working in the field of neurodegenerative disease by disemminating research findings to aid global research efforts to develop therapies for neurodegenerative disease; ii) Clinical colleagues who have taken our pre-clinical findings forwards into clinical trials (e.g. Trial testing Terazosin in motor neuron disease patients) in order to help refine and interpret trials and trial data; iii) patients on clinical trials receiving therapies we have previously identified (e.g. Terazosin) by refining and improving clinical trials.

Medium-Long Term (5-10 years) = Patients in the UK and global neurodegenerative disease communities by delivering new and effective therapies

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

We have an extensive network of colleagues and collaborators across the UK and global, research, clinical and patient communities. We are therefore ideally placed to disseminate the findings from this project (both positive and negative outcomes, as well as technical and methodological insights) across all relevant stakeholders.

Species and numbers of animals expected to be used

- Mice: 10000

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 required for these experiments for several reasons. First and foremost, it is simply not possible to model and study the complex cellular arrangements and networks that exist in the mammalian nervous system using in vitro or in silico systems. Thus, the ability to study neurons in their native environment in vivo is required in order to obtain data that is biologically-relevant to human neurodegenerative diseases. Secondly, human post-mortem material is not suitable for many of these studies as it is very difficult to obtain and, by its very definition, only provides insights into the very end stages of any disease. Whilst this stage is of some interest to researchers, our ability to delay or block the progression of neurodegeneration is going to rely on us being able to identify the triggers and early pathological changes for any given disease. Thirdly, mice are sufficiently complex mammals to model many important aspects of the human response to such diseases, including responses to novel treatments. Other animal models in lower

organisms do not reproduce this complexity. The complete genome for mice is available, speeding up the generation and availability of genetically-modified models that accurately reflect the human diseases.

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

Most animals will have genetic alterations that cause them to model a human neurodegenerative condition (for example, motor neuron diseases such as amyotrophic lateral sclerosis [ALS] or spinal muscular atrophy [SMA]). These animals can be used to study the natural course of the disease in the body, from stages before the disease has even generated symptoms, right through to stages where the main patient symptoms are recapitulated in the animal. This allows us to better understand how, where and why these diseases occur. It also then allows us to identify and test new therapies with the potential to treat neurodegeneration. As a result, some animals will receive experimental therapies, such as an injection of gene therapy or a new drug. Where appropriate, we may also be able to test the additive benefits of combining more than one therapy for a given disease (e.g. delivering both gene therapy and a new drug).

Animals will be monitored using a range of techniques (including some standard behavioural tests such as examining their ability to move and walk), with humane endpoints being carefully monitored to ensure that no animal undergoes extensive suffering that exceeds the severity limits of the licence.

Mice will be culled in order to obtain experimental tissue that can be used to study the biology of the disease, and/or impacts of a new treatment.

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

The majority of animals covered by this licence are simply required for complex breeding programmes to generate experimental animals. As such, they will not show any adverse effects.

For experimental animals that have been generated to model a specific neurodegenerative condition, they will display symptoms associated with the relevant disease. For the majority of experiments on this project, this will be animals displaying symptoms of motor neuron disease. These symptoms can include progressive muscular weakness (particularly affecting the hind limbs), weight loss, inability to move freely, hunched posture, reduced ability to respond to external stimuli. We have extensive experience of monitoring these adverse effects and have robust scoring systems in place to ensure that animals are culled before they experience excessive suffering.

Some experimental animals will also undergo interventional procedures that can lead to adverse effects. For example, animals can react to anaesthesia required for surgery and delivery of therapies (e.g. injections into a major vein of the body). Others may react in an unexpected way to a new therapy being delivered. Short-term effects such as post-operative/injection pain (usually lasting 24-48 hours) are handled by delivering pain relief to the animals. More significant and/or long-term effects will be detected through regular monitoring and can lead to immediate culling of the animal, if required, to minimise undue 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)?

Only around 20% of all the animals under this project will experience a severity greater than sub- threshold, due to the majority simply being a requirement for breeding programs rather than being experimental animals. Many of the experimental animals will be within mild severity limits (~10-15%). The remaining 5-10% of animals will be maintained within moderate severity limits. A very small number of animals (~1%) will fall under the severe severity limit due to extensive by temporary hindlimb paralysis that can occur as part of the process of modelling one specific form of spinal muscular atrophy (SMA). These mice are monitored very closely, and if the hindlimb symptoms don't improve over time (as predicted), can be culled immediately.

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

- Killed

A retrospective assessment of these predicted harms will be due by 08 March 2029

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?

Mice are required for these experiments for several reasons. First and foremost, it is simply not possible to model and study the complex cellular arrangements and networks that exist in the mammalian nervous system using in vitro or in silico systems. Thus, the ability to study neurons in their native environment in vivo is required in order to obtain data that is biologically-relevant to human neurodegenerative diseases. Secondly, human post-mortem material is not suitable for many of these studies as it is very difficult to obtain and, by its very definition, only provides insights into the very end stages of any disease. Whilst this stage is of some interest to researchers, our ability to delay or block the progression of neurodegeneration is going to rely on us being able to identify the triggers and early pathological changes for any given disease. Thirdly, mice are sufficiently complex mammals to model many important aspects of the human response to such diseases, including responses to novel treatments. Other animal models in lower organisms do not reproduce this complexity. The complete genomes for mice is available, speeding up the generation and availability of genetically-modified models.

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

It is simply not possible to model and study the complex cellular arrangements and networks that exist in the mammalian nervous system using in vitro or in silico systems.

However, wherever possible, we will use in vitro systems (e.g. to establish the action of a new compound on a given gene or protein target in fibroblasts), or lower model systems (e.g. Drosophila and/or zebrafish), to validate our experimental findings, rather than requiring additional mice . We also aim to validate our findings in human postmortem material (and/or iPSCs) wherever possible, to ensure that the neurodegenerative pathways we are working on in animal models are relevant to the human disease.

Why were they not suitable?

Whilst in vitro systems, and lower model animals (e.g. Drosophila and/or zebrafish) can be very powerful tools when used in combination with mammalian model systems, it remains the case that it is simply not possible to model and study the complex cellular arrangements and networks that exist in the mammalian nervous system without using a mammalian model. This is key for studying the complex nature of neurodegenerative diseases in vivo.

A retrospective assessment of replacement will be due by 08 March 2029

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 estimated numbers are based on the actual usage of animals over the past three PPLs. The vast majority of animals will simply be required for complex breeding programmes required to generate experimental animals with the correct genetic modifications to model a human neurodegenerative condition.

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

The main contributing factor to reducing the numbers of animals required for experiments is our prior expertise in working with the models and techniques covered by this licence. For example, we already have robust base-line data for key disease parameters (e.g. survival, body weight, neuromuscular function, neuromuscular pathology, biomarker changes etc) for most of the models being used. This means that experiments can be planned to obtain the maximum amount of robust, biologically-relevant data from the minimum number of animals, where comparisons to baseline datasets can reduce the amount of repitition required.

The group size for each new experiment is guided by our past experience, alongside power-analyses based on pilot data (using software such as ‘Power & Precision’

(http://www.power-analysis.com/).

Although we do have considerable statistical expertise in-house where required we also consult external expert statisticians. In our previous experience (and based on statistical power calculations), group sizes tend to range from 5-20 animals, with groups >8 only usually required when performing an extensive analysis of the potential therapeutic benefits of a new treatment (e.g. trialling a new neuroprotective compound).

All experiments are designed to conform to the ARRIVE guidelines (2.0).

The applicant and colleagues are actively involved in the international neurodegeneration research community, including regular contributions to research conferences, sitting on grant panels and belonging to editorial boards of leading journals. Therefore, we are well-placed to know what activities are going on in other research labs who are addressing similar research questions to us, allowing us to avoid duplication of effort (and use of animals).

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

The research team always maximises the use of tissues from each experimental animal. As such, we often run multiple different experimental assays by harvesting a wide range of tissues from each animal after culling. We also regularly supply tissue samples to collaborators in order to reduce their requirements to generate their own experimental animals.

Wherever we are undertaking a new experiment (e.g. trialling a new treatment for the first time) we perform pilot studies that allow us to better design subsequent full-scale experiments (e.g. calculating sample sizes, optimising assays etc).

We always follow best practice guidelines for breeding, and regularly update our policies and procedures (e.g. genotyping protocols) in order to minimise animal numbers.

A retrospective assessment of reduction will be due by 08 March 2029

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 models we have chosen to use (primarily genetically-altered rodents modelling neurodegenerative disease) are based on their close genetic and symptomatic match to the corresponding human condition. We preferentially use models that are established and accepted within the research community and that have a defined disease progression, allowing us to adhere to the severity limits of the license for each individual experiment and minimise the need for exploratory experiments to define baseline characteristics. For any new genetically altered lines brought onto the licence, we will always try to establish collaborations with those who developed the model (or have used it previously) to ensure that experimental design is based around current knowledge of the animals and their predicted phenotypes. Wherever possible, we choose to use models that have a shorter (rather than more prolonged) disease time-course, in order to minimise potential distress.

For the invasive procedures we may need to use (e.g. delivery of therapeutic substances by injection, behavioural tests etc), we have considerable experience of refining the methodologies as part of our previous PPLs. For any new techniques we routinely seek assistance from established collaborations to provide all required training and support.

The only protocol under this PPL catagorised as ‘severe’ is required in order to establish the potential benefits of combinatorial (e.g. “poly-pharmacy”) treatments for neurodegeneration in conditions such as spinal muscular atrophy [SMA]. This is based on a clear clinical need identified in recent clinical trials on human SMA patients, where one treatment alone was effective but insufficient to “cure” the patients. A ‘second generation’ of therapies will therefore be required, combining SMN-targeted therapies that are already available with additional (likely non-SMN targeted) therapies in order to generate a more robust, long-lasting impact on the disease. In order to develop and test this second generation of therapies, we need to be able to perform pre-clinical studies in mouse models of SMA that mimic human patients who have received SMN-restoration therapy where the treatment remains short of a 'cure'. The creation of such an ‘intermediate’ mouse model of SMA has already been reported by several groups. These intermediate models combine established genetically-modified mouse models of SMA, already used routinely in our laboratory, with sub-clinical/low doses of treatments that restore SMN protein levels. The higher severity limit for these animals is required due to the potential for some of these animals to display a hind limb weakness/paralysis phenotype (often transient, lasting for 24-48 hours.

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

Mice are required for these experiments for several reasons. First and foremost, it is simply not possible to model and study the complex cellular arrangements and networks that exist in the mammalian nervous system using in vitro or in silico systems. Thus, the ability to study neurons in their native environment in vivo is required in order to obtain data that is biologically-relevant to human neurodegenerative diseases. Secondly, human post-mortem material is not suitable for many of these studies as it is very difficult to obtain and, by its very definition, only provides insights into the very end stages of any disease. Whilst this stage is of some interest to researchers, our ability to delay or block the progression of neurodegeneration is going to rely on us being able to identify the triggers and early pathological changes for any given disease. Thirdly, mice are sufficiently complex mammals to model many important aspects of the human response to such diseases, including responses to novel treatments. Other animal models in lower organisms such as zebrafish and drosophila do not reproduce this complexity. The complete genome for mice is available, speeding up the generation and availability of genetically-modified models.

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

All animals will be monitored regularly by experienced animal house staff and members of the research team. All staff are familiar with the normal appearance and clinical signs in the animal models being used, and we have robust reporting procedures to ensure that any animals for which there are welfare concerns are notified to the PPL holder and veterinary staff as quickly as possible.

For the invasive procedures we have chosen to use (e.g. delivery of substances), we have considerable experience of refining the methodologies as part of our previous PPLs, including the administration of post-operative analgesia, where appropriate. For any new techniques we routinely seek assistance from internal or external colleagues with prior experience of the technique to provide all required training and support.

All behavioural tests to be used are already established in the laboratory and have proven to generate clear data correlating with neurodegenerative phenotypes (e.g. mice with SMA get progressively worse at the righting test and other motor function tests as the disease progresses).

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

We keep up to date with guidance from, and follow best-practice guidelines, from the following sources:

Home Office / UK Government (ASRU) website

ARRIVE 2.0 guidelines

UKRI's policy on use of animals in research

University internal guidance documents and training programmes

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

The applicant and colleagues are actively involved in the international neurodegeneration research community, including regular contributions to research conferences, sitting on grant panels and belonging to editorial boards of leading journals. Therefore, we are well-placed to know what activities are going on in other research labs who are addressing similar research questions to us, allowing us to avoid duplication of effort (and use of animals), and to be at the forefront of knowledge concerning advances in the 3Rs. In addition, the University provides regular training and update sessions on the 3Rs to all PIL and PPL holders.

A retrospective assessment of refinement will be due by 08 March 2029

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. Safety pharmacology and pharmacokinetics/toxicokinetics of pharmaceuticals in the non-human primate

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Safety, Pharmacokinetics, Pharmacology, Non-Human Primate, Toxicokinetics

Animal types	Life stages
Cynomolgus macaques	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

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 will allow for safety and pharmacokinetic tests to be conducted in non-human primates (NHPs) (Cynomolgus macaques) on pharmaceuticals, bio-pharmaceuticals and biologic drugs for use in humans for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life- threatening clinical conditions or their effects in man. It will also be required for associated research and development studies to, for example, gain comparative data and critically evaluate new test methods, equipment and techniques which may give better data. These development studies may be required to validate the animal model, generate background and/or reference compound data and therefore must

be performed in the same species as the intended pharmaceutical testing, in order to fully support new drug applications. The project aims to make sure that drugs going into clinical trials in patients don't have any side effects on the heart or the circulation, and there is enough of the drug in a patient's system to allow it to have its effect.

These studies will provide data to satisfy regulators around the world that these drugs are safe for use. These drugs will be used to treat debilitating human illnesses (like cancer and diabetes for example) for which there is an unmet clinical need, or the need for more effective drugs. The majority of tests on this licence are being carried out to meet global regulatory requirements (tests that governments require before they allow testing in humans).

No cosmetic products or chemicals that are exclusively intended to be used as ingredients in cosmetics will be tested.

A retrospective assessment of these aims will be due by 22 March 2029

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?

New medicines have the potential to benefit in new or improved disease treatments. Before potential new medicines are administered to humans their safety must be evaluated. This testing is a mandatory legal requirement and provides information on risks to people taking new medicines. Often, the new medicines we test on this programme will be highly specific for a molecular target or receptor, which often make them less likely to have side effects than traditional medicines.

At present there are no alternatives that don’t use animals that are scientifically, ethically or legally acceptable in these tests. In addition, we only use NHPs when no other species is suitable based on the nature of the drug (so if a drug can be tested in say a rat or a mouse, or in a dog or a pig, and we would expect an equal outcome to the study then we would use them instead of a primate).

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

The overall benefit of this project is that it supports the development of safe, new medicines to improve the health and quality of life of human patients by generating high quality data that is acceptable to regulatory authorities and enables internal decision making (i.e. whether drugs are safe to be tested in humans) within our client's organisations. Achievement of the objectives of this licence will enable safe development candidates to progress and will also help to remove unsuitable candidates from the development pipeline at an early stage, thus saving animals and resources. Drugs that have the potential to affect the rhythm of the heart, for example, are potentially fatal.

Study reports will be included in regulatory submissions to allow regulatory authorities to make judgements on whether to permit clinical studies or to licence a drug. Global guidelines recognise that the justification for animal-based safety testing is the need for regulatory authorities to have sufficient information to assess the risks to which humans are exposed to by new drugs.

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 safe, new drugs that help alleviate human conditions. 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.

One of the key benefits is the production of data that is required by regulatory authorities, to ensure medicines can be dosed safely to humans. These drugs that will be tested are for debilitating or life threatening human conditions, in some cases where there is an unmet clinical need to treat such conditions.

In addition, the models on this project may be used to assess the safety or other in life properties of a new drug and find a dose that causes no effect. This is important when planning future trials in humans, to make sure any starting dose in a clinical trial is safe for the patients taking it.

Our customers will also benefit, as the data we generate will allow them to progress their new drugs into clinical trial, or otherwise if they are found to have adverse side effects.

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 regulatory purposes or to support regulatory purposes (e.g. to support drugs progressing to clinical trials). Previously however, we have collaborated with customers and shared data we have produced in the form of Scientific publications that are in the public domain.

Species and numbers of animals expected to be used

- Cynomolgus macaques: 200

Predicted harms

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

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

We are using adult non-human primates on these studies. We only use non-human primates when other species (like rats, mice, dogs and/or pigs) are unsuitable to get the answers we need from the studies.

This is often because for the type of pharmaceuticals we are testing (for example 'biologics', peptides or antibodies) we can only see any toxic effects if we use primates, maybe because the biological target of the drug is only present in a primate, or maybe because the requirements of the study mean we can only use primates to get the results we need that will satisfy global regulatory bodies.

We are not allowed to use primates by law unless there is no other animal we can use that will give us the results we need to satisfy the regulatory authorities.

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

Animals are often habituated to procedures prior to dosing. This may include the use of facemasks, restraint chairs, telemetry jackets and other pieces of equipment we may use. During studies animals are often restrained for dosing and sampling (manual short term restraint with the exception of intravenous and inhalation dosing). This is to ensure that animals are familiar with equipment prior to dosing and recording, and the use of the equipment doesn't effect the parameters we are trying to measure in these studies.

On these studies, animals will be dosed with test materials (potential human pharmaceuticals) by a variety of different routes (including by injection and via inhalation by a special fitted mask) and undergo various tests to look at the effects of these drugs in the animals' system. This may include a period of restraint to aid dosing and recording for example. This will include taking blood samples from the animals from a vein (as a patient would give a blood sample to test their blood) to see how much of these test materials are present for example. Or sometimes in animals that have been implanted with a device to allow the measurement of blood pressure, heart rate and ECG, to look at the effects of drugs on those parameters. These tests are key to help determine if potential new drugs are safe for first use in man. Animals may be restrained for certain procedures such as blood sampling, but this only causes mild temporary distress, and usually on the first few occasions.

Most animals are expected to experience only mild effects such as slight weight loss. The doses used in these studies will be based on data gained in preliminary studies using small numbers of animals. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss or reduced activity.

Animals will be carefully monitored for clinical signs or other effects on their health and wellbeing, and in order to prevent unnecessary suffering, humane endpoints are applied under appropriate veterinary guidance (e.g. treatment with the test substance will be stopped or supportive or therapeutic treatments will be given to help the animals recover).

Food and water maybe withdrawn for a short a period of time, prior to specific investigations. These would include prior to surgery to allow blood sampling for clinical chemistry investigations (to ensure the animal is in good health), or prior to oral or inhalation dosing to a test material, if required (often this is not required). Animals will only have food and water removed when when scientifically justified and for the shortest time possible to meet the aims of the study.

Other rarely performed procedures include temperature measurement (using a rectal probe or nasal device), and single housing (mainly for recording of cardiovascular

parameters-to prevent cross contamination and to prevent interference with jackets on other animals.

Animals may undergo surgical procedures on up to 3 occasions, in order to implant/repair a telemetry device or implant a vascular access port. 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 veterinary surgeon before we start. We administer drugs as necessary and give them plenty of time to recover from surgery before we use them in experiments. These surgical procedures are carried out only for essential purposes. Animals in surgical studies may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients; for example, in the case of cardiovascular telemetry studies (where animals receive an implanted device), animals may experience post-operative pain/distress and possible infection. However, supportive treatments are given to eliminate or minimise these adverse effects. This may include providing extra warmth or special food.

Animals may be re-used if approved after a thorough examination by a veterinary clinician. If the animals are not approved for re-use by a veterinary clinician, they may be humanely killed at the end of a study. Using animals again is a good way of reducing the numbers we have to use in the first place.

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 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 deeply 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 and hold a UK personal licence outlining their competency in the procedure.

Generally, if we have to use any equipment to help us get the results we need, we acclimatise our animals to it so they get used to it and tolerate the procedure when we start dosing them. So, we carefully introduce them to things like restraint gradually, for short periods at first, and usually they accept it after a while. And if they don't acclimatise, we take them off the studies, to stop causing any harm.

Animals in surgical studies are normally regarded as experiencing moderate adverse effects (though they are given appropriate pain relief medication) and may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients, such as weight loss and infection (rare). However, supportive treatments are given to eliminate or minimise these adverse effects, and humane endpoints are again applied. All surgical procedures are performed under anaesthesia, with full peri- and post-operative analgesic cover to reduce/eliminate as far as possible any pain or discomfort during surgical recovery, as would be the case for a human patient. Animals that undergo more than one (up to a maximum of three) surgical procedure are not considered to experience any greater adverse effects than for the initial surgical procedure. Veterinary advice will be sought prior to each surgical procedure.

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

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

On the last project, about 10% of animals were classified as having experienced mild severity, around 90% were classified as moderate. The moderate severities in the last project would have been due to treatment-related signs of moderate severity (rarely due to previous studies in the same species) or because a surgical procedure was involved. Surgically prepared animals are deemed to have experienced Moderate severity under the law regulating these studies.

It is impossible to predict the proportion of severities expected on a service licence like this, as this will be dependent on what study types we are asked to perform. However, a distribution between 'mild' and 'moderate' severities similar to those in the last project are anticipated.

All protocols on this licence are classified Moderate only, there is no intention to perform any procedures that are Severe in nature. Under the last project licence (at the time of writing) no animal had been classified as having experienced severe severity.

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

- Killed
- Kept alive
- Used in other projects

A retrospective assessment of these predicted harms will be due by 22 March 2029

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 many validated non-animal or in vitro tests available to examine the effects of test substances on specific cellular processes. There are, however, as yet no non-animal tests or in vitro test systems available that can fully replicate the effects of substances on the complex biological interactions that occur between the various cells, tissues and organs that constitute a living organism.

We would only use primates in these studies because the regulators demand we use more than one type of animal species (other than say a mouse or rat) to confirm that drugs are safe. Normally we would use something like a dog or a pig for this purpose, but sometimes this is not possible due to the specific type of drug that is being investigated, and the target

it hits, which may not be present in dogs or pigs. Its only then we'd use primates, and only after there had been specific justification to do so.

These tests are also mandatory for all drugs prior to use in humans (clinical trials and in clinical use in patients).

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.

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

Some of the testing related to this licence can be done in vitro (test tubes). There are cells that contain the IKr channel contained in the NHP, and in some instances, this test will be able to satisfy regulatory authorities regarding the risk of any putative pharmaceuticals for human administration.

These tests are carried out on a case-to-case basis using a risk assessment for safety in humans (based on similar chemical structures), using a stepwise testing paradigm. In many cases, taking all things into consideration, testing in animals would still be a preferred option for global regulators, to ensure safety in humans

Why were they not suitable?

Although there are test tube tests that can model some parts of how drugs get into our bodies, and how our body deals with them, and can identify undesirable effects, for example, there is no series of test tube tests that brings all these complex happenings together, like we see in animals and humans.

That's why we need to test the new drugs in animals, as they have similar physiology and processes as humans, and that testing gives us a good idea what may happen if they were ever tested in or exposed to humans.

However, in all cases we will assess whether data already exists or can be generated in other ways other than the use of animals, and that we will ensure that animal reduction, replacement or refinement strategies and alternatives provided in the regulatory guidance will be considered, and animal use avoided where possible.

A retrospective assessment of replacement will be due by 22 March 2029

The PPL holder will be required to disclose:

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

Reduction

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

These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

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

The numbers we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received in the past. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

The numbers of animals used in each study are in some cases specified in the regulatory guidelines; where not specified, numbers are based on established minimum regulatory expectation, or on scientific estimates of the minimum numbers required to meet study objectives.

All experiments will be designed in order to achieve the scientific objectives (to see that drugs get into the system so they work, and checking that they don't affect the heart and circulation) using the minimum numbers of animals. Because our experiments are run to satisfy regulators that drugs are safe, and we have a lot of experience in performing them over many years, so we know what numbers give the right sort of data the regulators require. Statisticians will be consulted to assist with group size and dose design.

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.

For regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international guidelines where these exist. Otherwise, reference is made to standard study designs with input from the Department of Statistics, where appropriate, to identify the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. These internal designs are reviewed and updated in line with changing external guidelines and internal refinements that either minimise numbers or reduce severity.

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.

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.

The re-use of animals, under strict legal guidance and the approval of a veterinary surgeon who is aware of the lifetime experience of the animal, also reduces the overall numbers of animals used on this project.

A retrospective assessment of reduction will be due by 22 March 2029

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 use adult non-human primates (Cynomolgus macaques). Non-human primates will only be used when they are scientifically the only appropriate species for a particular study and no other species (like the pig or dog) or non-animal alternative is available or acceptable to achieve the objectives of the study.

The models we use are the least invasive procedures, for the least amount of time we need to do them, to get the information we want. They are carried out using standard and recognised techniques by fully trained staff. We also have veterinary help on hand for advice and on the occasions we have to anaesthetise the animals.

Dosing and sampling procedures will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than short term discomfort and no lasting harm and will be the minimum consistent with the aims of the studies. This will often be similar to an injection you may receive at the doctors or in hospital, or maybe like having a blood sample taken in a doctor's surgery. In addition, any suffering will be further minimised by implementing clearly defined humane endpoints. So, if anything happens to adversely affect the animals, they will be humanely killed whether the experiment is finished or not (this is very rare).

For situations involving restraint procedures (e.g. in a chair or in a metabolism cage) the animals are habituated to this equipment starting with short periods, then building up. Most animals habituate fine to this equipment, but if they don't (rare) we remove them from the study. This is critical to the quality of data produced (especially cardio-respiratory data) and means that the animals are prepared for the dosing phase of the study.

For the management of animals undergoing recovery surgery, an aseptic approach will be used. These surgeries will be carried out by veterinary surgeons. Appropriate pain relief will be provided as required. Surgical procedures will be carried out in accordance with the principles set out in the LASA (laboratory Animals Science Association) Guiding Principles

for Preparing for and Undertaking Aseptic Surgery (2017). This is the same sort of care that a patient in hospital would receive when undergoing an operation.

Care is taken to provide as much environmental enrichment as possible, and working parties that often test and introduce environmental enrichment e.g. introduction of specific between feed snacks for primates based on their preferences. Animals also have toys to play with, have food scattered on the floor of their cages so they can forage as in the wild, and have access to visual stimulation (TV screens) for example.

Animals will be housed in social groups. Animals will only be singly housed where study related procedures require, for example telemetry recording involving external jackets (to prevent interference with cage mates jacket), to minimise potential for cross-contamination or for welfare reason (e.g. recovery from surgery). The duration of single housing will be kept at a minimum to fulfill the study aims.

Food and water maybe withdrawn for a short a period of time, prior to specific investigations. These would include prior to surgery to allow blood sampling for clinical chemistry investigations (to ensure the animal is in good health), or prior to oral or inhalation dosing to a test material, if required (often this is not required). Animals will only have food and water removed when when scientifically justified and for the shortest time possible to meet the aims of the study.

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

Non-human primates are only used when no other species is suitable to get the information we need. In fact, we have to prove that the primate is the only species that will give us the answer we need (instead of rodents or dogs or pigs) that will translate to the effect we would see in man.

Most of these studies require dosing to assess potential adverse effects in man, so it is not practical to perform them under terminal anaesthesia, as in many cases, the use of anaesthesia will make interpretation of the results more difficult and give a less definitive answer.

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

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet at least the standards set out in the LASA (Laboratory Animal Science Association) 2017 guidelines for Aseptic Surgery. Before we start surgery, we agree with a Vet what pain killers or antibiotics the animals need both before and after the surgery. When recovering from surgery, we give the animals extra heat and monitor them closely until they start behaving normally again. We then check them at least twice daily before they go on study, and they have plenty of time to recover (weeks not days). In the event of post-operative complications, animals will be killed unless, in the opinion of the Named Veterinary Surgeon, such complications can be remedied promptly and successfully using no more than minor interventions (unless further investigations are permitted in this licence).

We have introduced a newly designed restraint chair for animals which provides sufficient restraint but allows more natural movement, and comfort. We use this type of restraint

during infusion and inhalation dosing and the period of time the animal is restrained in the chair is limited on a daily basis.

During dosing and restraint, animals are constantly and closely watched for signs of distress.

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 a veterinary surgeon is on call at all times to assess and relieve any adverse events.

Refinements to improve the animals experience include but are not limited to group housing, environmental enrichment, including novel toys and foods, human interaction, acclimatisation and training to procedures, to move around the cage and to leave the cage voluntarily as required, forage opportunity and calming measures such as stroking/gentle talking are used to help animals have a better experience of restraint. The use of upgraded telemetry recording systems and transmitters (used in the majority of the studies performed under this project) means that animals are not routinely singly housed for recording of cardiovascular parameters anymore.

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

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).

ICH Guideline S7A. Anon 2000. Committee for Proprietary Medicinal Products (CPMP). Safety Pharmacology studies for human pharmaceuticals. The European Agency for the evaluation of medicinal products. London, November 16, 2000. Reference CPMP/ICH/539/00.

ICH Guideline S7B. Anon 2005. Committee for Proprietary Medicinal Products (CPMP).The non- clinical evaluation for the potential for delayed ventricular repolarisation (QT interval prolongation) by human pharmaceuticals. Reference CHMP/ICH/423/02.

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/

Diehl et al. 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).

https://www.nc3rs.org.uk/3rs-resources/blood-sampling A Guide to Blood sampling in common laboratory species

ICH S3A Guideline. NOTE FOR GUIDANCE ON TOXICOKINETICS: THE ASSESSMENT OF SYSTEMIC EXPOSURE IN TOXICITY STUDIES S3A . Anon. 1994
ICH S3B Guideline. GUIDANCE FOR REPEATED DOSE TISSUE DISTRIBUTION STUDIES S3B.
Anon 1998

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.

A retrospective assessment of refinement will be due by 22 March 2029

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. Prevention and control of coccidiosis in poultry

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
  - 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

Efficacy, Safety, Parasites, Coccidia, Poultry

Animal types	Life stages
Domestic fowl (Gallus gallus domesticus)	juvenile, adult, neonate
Quail (Coturnix coturnix)	neonate, juvenile, adult
Turkeys	neonate, juvenile, adult

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

The overall aim of the programme of work is to provide efficacy and safety data for products for the control and prevention of coccidiosis in poultry. Coccidiosis is a protozoan

disease caused by various species of the genus Eimeria. The disease is characterised by enteritis, diarrhoea and mortality.

Coccidiosis is the most prevalent disease affecting the broiler industry. Subclinical cases will hit profitability through poor feed conversion, while damage to the gut wall makes it easy for more virulent challenges to take hold, like necrotic enteritis. Parasite control products are continually being developed, but it is a legal requirement for these to be fully tested for safety and efficacy prior to them being marketed. This licence will enable studies to be carried out on behalf of pharmaceutical companies to satisfy these legal requirements. Continuous and long-term use of infeed ionophore coccidiostats has resulted in resistance and less effective control of coccidiosis. Resistance results in impaired performance, particularly poor weight gain. Work is being carried out to evaluate new active substances as coccidiostats and inbitiors, as well as evaulating combinations of existing actives and renewal evaulations of exisiting actives.

Additionally vaccines are being developed with the aim of combating resistance and increasing the ease and improving cost of treatment.

Alternative vaccines are being developed as alternative to the use of live vaccines which are difficult and costly to produce and rely on parasite recycling in birds for the stimulation of full protection. The design of modern poultry facilities does not provide ideal conditions for recycling of vaccinal oocysts which can result in a delay in the onset of immunity and protection to the birds.

Additionally feed additives are being investigated to aid in the prevention of coccidiosis and resisilence of the bird to coccidiosis as supplements to pharmaceuticals routes of control.

A retrospective assessment of these aims will be due by 19 March 2029

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 overall aim of the programme of work is to develop safe and effective means of controlling coccidiosis in poultry. Disease and ill health caused by coccidiosis in poultry continues to be a worldwide welfare concern. This problem is being exacerbated by the rising levels of resistance to various products.

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 coccidiosis.

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 coccidiosis in poultry. 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, the consequences of this work not being carried out would be that safer, more effective products would not be available to treat coccidiosis in poultry.

The programme of work is restricted to the prevention and control of coccidiosis in poultry. Controlling coccidiosis in poultry is a worthwhile pursuit, as there are significant consequences associated with disease outbreaks, including animal welfare issues and economic losses. The global impact of coccidiosis due to decreased performance, morbidity, and mortality is an estimated $300 million US dollars (The Poultry Site Feb 2013). Subclinical cases will hit profitability through poor feed conversion, while damage to the gut wall makes it easy for more virulent challenges to take hold, like necrotic enteritis.

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

- Domestic fowl (Gallus gallus domesticus): 33000
- Quail (Coturnix coturnix): 500
- 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.

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

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

Animals may be included on safety or efficacy studies. These usually range from 3 to 10 weeks of duration. Birds will recieve the test product either by oral gavage, in feed or by injection. Faeces is collected for oocyst output, clinical observations are carried out daily, birds weighed and feed intake measured. For efficacy studies birds may be inoculated with the relevant Eimeria species. Birds will be humanely euthanased for assessment of gut lesion scores.

In order to carry out safety or efficacy studies it is also necessary to carry out dose titration studies or produce vaccinal or challenge material by passaging through naïve birds to produce fresh viable material for use on studies. Dose titration studies allow for optimisation of challenge dose rates to ensure correct dose rates are used on study to have sufficient clinical changes with lowest level of severity possible and to ensure assessments e.g. gut lesion scoring is carried out at the optimum time. Dose titration studies are typically smaller versions of the efficacy design with 10 birds per challenge rate and per lesion scoring time point.

Vaccine and challenge production requires naive birds approx 2-3 weeks of age, inoculated with the relevant species. Birds are placed on slatted flooring to ensure all faeces can be collected. Faeces is collected for approx 5 days after the pre patent period. Bird numbers will vary based on the species passaged, e.g. E. acervulina only requires approx 20 birds but E. maxima requires approx 90 birds.

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

Birds inoculated with pathogenic Eimeria may become ruffled, dull and have diarrhoea. Some mortality can be expected for birds inoculated with E. tenella and E. necatrix. Some weight loss is also expected with all species.

Birds not recieving pathogenic material are not expected to have any adverse effects of procedures

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)?

Birds on safety studies or vaccinal production would not expect to exceed mild.

Birds on efficacy studies, dose titration studies or challenge production involving the majority of Eimeria species would not expect to exceed moderate.

Birds on efficacy studies, dose titration studies or challenge production involving inoculation with E. tenella or E. necatrix would expect up to 50% of birds could be severe.

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 19 March 2029

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 19 March 2029

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 vaccines and coccidiostats.

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 19 March 2029

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 we propose to use are as dictated by European guidance documents. In many cases the adverse effects are likely to be mild or 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 the animal. Dose titration tests will be undertaken to ensure the minimum dose required is being used to reduce the severity of the challenge.

Birds will be housed in guidance with current welfare guidance, ensuring adequate space, appropropriate feed and the use of enrichments and lighting as applicable for the breed and age of the bird. Very young chicks especially game birds such as quail may initially be housed in rabbit hutches/cages, which ensures adequate space but prevents too much space when being caught and handled for procedures to reduce stress on the birds.

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 NTCOs and NACWOs to instigate throughout the establishment.

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 19 March 2029

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. Pharmacokinetic, pharmacodynamic and safety studies for companion animal therapeutics

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

Veterinary medicine, Monoclonal antibody, Pharmacokinetic, Pharmacodynamic, Safety

Animal types	Life stages
Beagles	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 benefit

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 assess the how well new treatments developed specifically for pet dogs remain detectable and/or functional over a defined period of time. From these studies we will also confirm how safe they are when given either once or multiple times. The information gathered here will inform us on which drug is best suited to take forward to obtain regulatory approval.

A retrospective assessment of these aims will be due by 26 March 2029

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 programme of work is essential to establish the safety and biological activity of potential new treatments within the target species. Cell based efficacy tests provide valuable information, however it is necessary to confirm the anticipated effect within the target species. The information gathered will lay the foundations to progress therapeutic candidates forward to clinical trials necessary to obtain market authorisation. The new therapeutics aim to address a range of indications such as cancer, atopic dermatitis, and pain.

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

Output 1: Pharmacokinetics

This work will allow us to establish how long a particular drug remains at a detectable level within a dog after it has been dosed at an established concentration. We can use this information to progress drugs with the most favourable characteristics.

Output 2: Pharmacodynamics

For some of our drugs we can tell what effects or actions they have on an animal. For example how effective they are at reducing a certain type of cell population. This information can be obtained from the same routine blood draws that are required for output 1.

Output 3: Safety

After giving a drug at a set concentration it is possible to check the blood composition to gain an insight into how safe the drugs are. It is not expected for the drugs to be harmful.

These outputs will inform us on which drugs are most suitable to progress through to pivotal clinical trials to obtain authorisation from the United states department of agriculture (USDA) and European Market Authorisation (EMA).

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

Short term: Monoclonal antibody therapeutics being tested in this license that prove to be safe and effective may progress through clinical trials within the field of clinical veterinary medicine so that companion animals can be treated in the UK under animal test certificate authority. The information gathered under this license will also contribute towards obtaining USDA approval so that our therapeutics can be used in the United States and European Market Authorisation to enable their use within Europe.

Long term: Achievement of the objectives of this licence will enable safe and effective therapeutics to progress towards obtaining marketing authorisation in the United States of

America and throughout Europe. The therapeutics will become available for use in clinical veterinary medicine to improve the health and welfare of our pet dogs, treating a range of potentially serious and debilitating illnesses, such as cancer and atopic dermatitis.

Without these studies, the progression of new medicines to pet dogs could not occur.

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

Therapeutic candidates tested under this license will become available as companion animal therapeutics for use in clinical veterinary medicine. Appropriate business development will result in these drugs being administered internationally.

Data obtained under this license will be published in the form of patents and it is expected to be published in scientific journals so that some of the knowledge gained under this license can be utilised by other research groups.

Species and numbers of animals expected to be used

- Beagles: 80

Predicted harms

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

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

We are developing a number of species specific (dog) therapeutic antibodies to address a broad range of diseases in companion animals (dogs). Adult Beagles accurately represent the target species that our therapeutics are designed to treat. Therefore, there is no better alternative species to achieve the aims of this project.

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

Administration of the test substance by directly injecting them into a suitable vein (intravenous), or by injecting them under the skin (subcutaneous). These represent conventional routes that we anticipate veterinarians will eventually use.

The collection of blood samples from a suitable vein before or after a test substance is dosed. Blood withdrawal is limited to 20 occasions over any 8-week period. The minimum sample size appropriate for the scientific purpose will always be taken.

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

The test substances are novel fully canine monoclonal antibodies. As such we do not anticipate significant immune reaction against it, however, because no canine antibodies exist at present that target the same proteins in dogs it is impossible to rule out any associated effects.

Human monoclonal antibodies which target some of the equivalent proteins in humans have been developed and show a very safe profile in both phase I and II clinical trials.

Despite the very safe profile, rare adverse effect such as vomiting or diarrhea cannot be ruled out and animals will be closely monitored in the hours following the administration of novel therapeutics.

Procedural impacts:

Performing subcutaneous or intravenous injections are not anticipated to produce any side effects; these routes are routinely used to administer therapeutics.

Repeated blood withdrawals are expected to cause mild discomfort that will subside very quickly after the procedure and have no lasting harm.

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)?

90% of animals under this license are expected to experience a mild severity. The remaining 10% may experience moderate severity.

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 March 2029

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?

It is a regulatory requirement to test novel dog therapies in laboratory dogs in a controlled setting before clinical trials in client-owned animals can be undertaken. In order for much needed new therapies for underserved conditions affecting pet dogs to be developed, there must be trials carried out in beagles in the way proposed in this license. The testing here can be viewed to have similarities with human Phase I testing – i.e., at some point in the drug development process it becomes essential to test in the target species.

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

When selecting the best antibodies produced from an immunisation campaign, we employ a ‘sequence-first’ approach meaning that we select antibodies based on certain known characteristics within their protein make up. By selecting for characteristics which are

predictive of the most desirable therapeutics we can reduce the number of possible antibody sequences from several thousand down to 100-300.

A large amount of work using cells has or will be conducted for each therapeutic candidate to reassure us that only the best and most likely to succeed candidates progress to studies under this license. For example, using an immortalised cell line to confirm the ability of each monoclonal antibody to perform the desired biological function in the target cell type. This allows us to produce better therapeutics which are likely to be more successful when entering dog studies under this license.

Unfortunately, at this point there are no non-animal alternatives.

Why were they not suitable?

Adopting a bioinformatic approach to cell screening is a very useful tool for excluding certain antibody sequences early on in a discovery process however it does not inform us on exactly how well the antibodies would perform in the whole animal. Equally the cell-based tests that we have performed, they are very informative but do not represent a whole animal that will have many complex interactions between different cells and organs. Something which simply cannot be recapitulated by testing cells.

A retrospective assessment of replacement will be due by 26 March 2029

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 required is related to the number of candidate therapeutics that we expect to progress from a selection of antibody discovery campaigns.

For each study type we expect to require around 20 dogs, this facilitates the use of up to 4-5 experimental groups that will allow us to test up to two lead candidate drugs or a range of concentrations along with a control group. Considering the expected number of reuses of each animal and the number of drug targets, it is reasonable to expect to use 80 animals for the duration of the project.

The number of Animals required also considers the probability of re-using the vast majority of animals between different target indications. Where animals are reused, they will normally remain naive to therapeutics addressing the same target.

In some cases pilot pharmacokinetic and safety studies will be conducted under another project license or at a contract research organisation using mice or rats before progressing candidates to dog studies. It is reasonable to infer the translation of bioavailability and pharmacokinetic data, at least by rank, thus reducing the number of Beagles required under this license.

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

Control groups provide an essential contrast to the drug treatment groups. They will provide critical baseline data that drugs can be assessed against. Beagles will be allocated randomly to experimental groups by using a randomisation tool.

Technicians and veterinary staff will also be blinded to the candidate therapeutics being administered as part of the experimental design strategy.

Stepwise progression through increasingly complex studies is common when testing novel drugs, with the intention of providing enough data and confidence in a candidate to progress it to pivotal clinical trials that can be used to obtain USDA or EMA.

For every study plan type the NC3R's EDA tool will be used to help validate and provide clarity on the structure of each study.

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

Precursor dosing studies will be completed in advance using mice and rats to help narrow down our best candidates and best routes of administration, this will result in only the best candidates advancing to this stage of assessment. Antibody therapies may also be engineered to exhibit more desirable criteria that may evoke more desirable characteristics.

The number of animals required also considers the probability of re-using the vast majority of animals between different target indications. Where animals are reused, they will be held over a period of time to account for a long wash-out period associated with monoclonal antibodies. This is expected to be ~7 times the calculated half-life. Beagles will normally remain naive to therapeutics addressing the same target.

A retrospective assessment of reduction will be due by 26 March 2029

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.

Beagles will be the only animals used during this project. All of the Beagles will only receive a therapeutic drug by a well-established route which is transferable to clinical veterinary medicine so that we can establish at a very early stage how our drugs behave. Therapeutics will be given by subcutaneous or intravenous injection, which are commonplace when giving pet dogs vaccinations or when giving some cancer therapies such as chemotherapy.

Blood withdrawals are crucial and will be taken at predetermined time points to understand how long our drugs remain active in an animal. This procedure is simple and well characterised, like our routes of administration, we will use the same techniques that are used in clinical veterinary work.

The therapeutics we plan to administer already have an equivalent drug in humans (which have FDA approval), the targets are well characterised so we can predict very few minor or transient adverse effects.

Beagles will undergo positive reinforcement training to habituate them towards being handled and the presence of experimental equipment, such as shavers or the presence of (capped) needles.
Control groups will be used to benchmark physiological condition.

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

Our therapeutics have already passed intensive in vitro screening processes and will also have been screened in rodents so that only the best and most likely to succeed candidates are taken forward into Beagles. Because we need to study how therapeutics behave in a healthy animal of the intended species. The therapeutics that will be tested will likely need to be given to patient animals every 1-3 months. We therefore need to examine how they behave and confirm that they are safe over equivalent periods of time.

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

Daily monitoring and scoring of the animal’s general condition will be performed using standard facility templates. Clinical observations will also be recorded as part of a welfare monitoring process.

Utilise the beneficial interaction that is well documented between humans and dogs to refine the lifetime experience of the dogs. This will include training, desensitisation and habituation with the aim of refining basic husbandry tasks and regulated procedures. Positive reinforcement training (PRT) will be utilised to help achieve this. One of the facility management team will lead PRT training of staff and maintenance of standards between technicians and veterinarians.

Enhanced training for technicians in being able to recognise aggressive signals and how to interpret them in order to reduce stress and negative behavioural interactions. Being able to identify both positive and negative welfare indicators will be essential to achieve this.

Carefully considered husbandry routines that are adapted in response to identifying any causes of conflict due to human interaction. CCTV may be used to monitor and assess behavioural traits.

Care staff and veterinarians will periodically review mechanisms in place to monitor and deal with signs of aggression.

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

Where appropriate we will act in best accordance with the PREPARE guidelines from norecopa/RSPCA particularly with regard to the equivalent methods sections.

The Experimental design assistance (EDA) developed by the NC3Rs will also be utilised to help validate study design and also to enhance understanding of overall study design by providing useful visualisations of experimental groups and commissions.

The NC3Rs good practice guidance documents will also be utilised when performing administrations and blood withdrawals.

The guidance provided in the document 'Refining dog husbandry and care' will be followed as far as is practicable and applicable.

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

Subscription to regular newsletters from groups like the NC3Rs, LASA and the RSPCA as well as attending relevant courses and conferences will help to stay informed on best practice. Close liaison with the Named Information Officer at the establishment will also facilitate efficient passage of information.

A retrospective assessment of refinement will be due by 26 March 2029

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?