Home Office

NON-TECHNICAL SUMMARY

The immuno-metabolic mechanisms of cardiovascular disease

Project duration

5 years 0 months

Project purpose

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

Key words

cardiovascular disease, immune system, Cardiovascular risk factors, pathophysiology, therapy

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

Heart and blood vessel diseases happen in two main ways. The first is when fatty deposits build up inside blood vessels, blocking them (this is called atherosclerosis); this can lead to heart attacks, heart failure, and strokes. The second is when the walls of blood vessels become weak or stretched, which can cause them to bulge (this is called aneurysm) or burst (this is called aortic dissection). We want to understand how well-known risk factors, such as high cholesterol, high blood pressure, and an unhealthy gut, interact with the body's immune system to affect how these diseases start and get worse. By understanding this, we hope to find better treatments.

A retrospective assessment of these aims will be due by 9 January 2032

The PPL holder will be required to disclose:

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

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

Why is it important to undertake this work?

Heart and blood vessel diseases are still one of the leading causes of disability and death around the world. There is growing evidence that our immune system (the body's defence against illness) plays an important role in these diseases. However, we still don't fully understand how this works, and this has held back scientists from finding new and effective treatments. We believe that by better understanding how well-known risk factors, such as high cholesterol, high blood pressure, and an unhealthy gut, affect the immune system and contribute to heart and blood vessel disease, we could completely change the way we treat patients who have, or are at risk of developing, these conditions.

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

This research will help us better understand how the immune system responds to fatty deposits in our arteries, and how it helps the heart heal after injury. We expect our findings to lead to better ways of tackling atherosclerosis (the blocking of arteries by fatty deposits) and its complications, such as heart

attacks and heart failure. We also expect to greatly improve our understanding of aneurysms, where artery walls bulge or balloon outward, and to identify key targets for new treatments. Our work will also look at the role of gut bacteria in heart and blood vessel disease. Finally, we will explore how being exposed to risk factors before birth (while still in the womb) may affect a person's chances of developing heart disease later in life. Our findings will be published in scientific journals and may lead to new patents.

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

Atherosclerosis (the build-up of fatty deposits inside arteries) is the main cause of most heart and blood vessel diseases, which remain the leading cause of death and disability worldwide. This includes conditions such as aortic aneurysms, where artery walls bulge and can burst, causing serious harm and early death. In the UK alone, millions of people live with heart and circulatory conditions. These diseases account for more than a quarter of all deaths and place a huge financial strain on the health service and society. The total yearly cost to the UK economy, including early deaths, long-term disability, and unpaid care, runs into tens of billions of pounds (BHF statistics compendium, https://www.bhf.org.uk/what-we-do/our-research/heart-statistics/heart-statistics-publications/cardiovascular-disease-statistics-2025).

Direct benefits we expect from this project in the near future:

We will develop better laboratory models to study heart and blood vessel diseases. These will be useful tools for researchers around the world. For example, we have already created a mouse model showing that early and repeated exposure to a high-fat diet can significantly speed up the development of atherosclerosis.

We will gain a deeper understanding of how atherosclerosis, heart attacks, and aneurysms develop and cause damage in the body.

We will identify new biological targets (specific molecules and cells) that could be used to develop better treatments for patients with heart and blood vessel diseases.

Who may benefit in the near future: this research will help scientists and doctors better understand diseases of the heart and blood vessels. This will mainly benefit patients at risk of heart attacks, strokes, and aneurysms, especially people with high cholesterol, obesity, or a family history of heart disease. By learning more about how these diseases start and develop, doctors will be better able to find them earlier and choose the right treatments. For example, this could be life-changing for a middle-aged person at risk of a heart attack, as doctors may be able to detect the disease earlier and prevent it before serious damage happens.

Longer-term indirect benefits:

We will improve our understanding of how specific immune cells and inflammation contribute to the start and progression of heart and blood vessel diseases.

We will advance new ways of identifying people at risk earlier, and of tracking how their disease is progressing, using the latest imaging and biological marker technologies.

We will help pave the way for more effective and targeted treatments for atherosclerosis, heart attacks, heart failure, and aneurysms. This will ultimately lead to better outcomes for patients and reduce pressure on the health system.

Who may benefit in the longer term: this research could lead to new and better treatments that target the exact cells and processes causing the disease. This means patients could receive more personalised care, helping them live longer and healthier lives. It could also help doctors identify people at risk much earlier, even before symptoms appear, so they can take action sooner and avoid serious events like heart attacks or strokes.

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

We work closely with a wide network of doctors and scientists, both in the UK and around the world. We exchange ideas, sometimes mouse strains, and work in a complementary manner to address one single research question from various but complementary angles, according to each scientist's expertise. I hold editorial roles (helping to review and select scientific articles) at many well-respected international scientific journals, and I am a member of leading scientific societies across the globe. Whatever we find (whether our results are positive or not) we will share them at international scientific conferences (we attend at least 2 international conferences annually), publish them in peer-reviewed scientific journals, and make them available to the general public (for example, through news outlets, and participation to science festivals).

Species and numbers of animals expected to be used

- Mice: 48000

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.

Most of our work focuses on heart and blood vessel diseases that develop during a person's lifetime, rather than those that are inherited or present from birth. For this reason, the majority of our experiments will be carried out in adult animals. However, in some cases we will use pregnant mice to study how risk factors during pregnancy might affect the chances of developing heart disease in offspring later in life.

We have chosen to work with mice for two main reasons. First, mice are well-established and widely used models for studying heart and circulatory diseases, meaning scientists have a lot of confidence in what we can learn from them. Second, a large number of genetically modified mice (mice that have had specific genes altered) are available to researchers. This allows us to study how particular genetic changes influence the development and progression of heart and blood vessel diseases.

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

In a typical experiment, a mouse will be fed either a normal or high-fat diet for 6 to 12 weeks. It may have blood samples taken fewer than three times during the experiment, sometimes after a short period of fasting of up to 16 hours. The mouse will be regularly health-checked throughout, and at the end of the experiment it will be given a deep anaesthetic (a medicine that causes a state of complete unconsciousness) and will be painlessly killed while in this state. The animal will not wake up before it is killed.

In some experiments, we will use mice that have previously been pregnant.

In another typical type of experiment, a mouse will undergo a procedure that causes a controlled injury to a heart blood vessel. It will then be regularly health-checked and scanned using safe, non-harmful imaging (this is ultrasound without administration of any imaging agents, and not requiring anaesthetics) to monitor how the disease develops, before being painlessly killed under deep anaesthesia at the end of the experiment. In a very few numbers of experiments (less than 5%), an agent that mimics an infection may be administered to the mouse to examine whether this may affect the heart response to injury. In this case, the experiment will typically be stopped within 48 hours after the injection of the infection-mimicking agent.

In another typical type of experiment, a mouse will undergo a procedure that causes a controlled injury to the aorta (the large blood vessel that carries blood from the heart)to induce aneurysm (bulging of the aorta). It will then be regularly health-checked and scanned using safe, non-harmful imaging (this is ultrasound without administration of any imaging agents, and not requiring anaesthetics) to monitor how the disease develops, before being painlessly killed under deep anaesthesia at the end of the experiment.

In most experiments, the total number of procedures carried out on any one animal is fewer than five.

In a worst-case scenario, up to three additional procedures may be added. These could include whole-body radiation followed by a bone marrow transplant via an injection into a vein (used to better understand how the immune system contributes to disease), injections of substances that alter gene activity or modify the disease process, or short periods of single housing with or without some food restriction, to study how the body processes and uses food.

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

Some genetically altered mice will be bred to model inherited cardiovascular disease and may develop harmful phenotypes as a result of their genetic modification. These may include skeletal abnormalities, impaired mobility, or severe cardiovascular complications, meaning that some animals may require humane euthanasia or may die prematurely due to the underlying phenotype.

Some procedures in our experiments may cause discomfort or side effects in the animals. These can include pain after surgery (which may cause a mouse to hunch over, move less, or become still) or discomfort caused by a heart attack, an aneurysm that is close to bursting, breathlessness, or weight loss linked to heart failure.

In most cases, these effects are expected to last only a few hours. Animals are checked regularly so that we can reduce or treat any discomfort, for example by giving pain relief medication, or by ending the experiment early and humanely killing the animal before its condition gets worse.

In some experiments, animals may experience more serious discomfort. This is mostly a direct result of the disease being studied; for example, severe breathlessness or significant weight loss from heart failure, or signs of infection-related illness such as loss of appetite, dehydration, and reduced movement. In these cases, we may allow these effects to last up to 48 hours, because understanding exactly how and why they occur is the whole point of the research (with the ultimate goal of finding treatments that could help patients). In all such cases, animals are checked several times a day and will be humanely killed before they reach the maximum level of suffering permitted in the experiment.

In some experiments, animals may die suddenly during the course of the experiment. In most cases, this is due to the disease being studied (an aneurysm that ruptures). We have identified imminent signs of sudden death and have put in place measures to closely monitor the mice (every 2 hours) to detect signs of distress suggestive of rupture and kill the mice humanely before rupture actually occurs.

We will ensure that the combination of several steps under a given protocol will be kept to the minimum possible. In particular, an animal will not undergo more than 3 optional steps that may cause more than transient harm or distress. Moreover, no animal will undergo a step that may cause more than transient harm or distress if they have not already recovered from a previous step.

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: 29000 x 0.9 = 26100 = 54.4%

Mild: Protocol 1 = 500; Protocol 2 = 500 x 0.5 = 250; Protocol 4 = 29000 x 0.1 = 2900; Protocol 5 = 7500 x 0.385 = 2888; Protocol 6= 6000 x 0.8 = 4800; total = 11338 = 23.6%

Moderate: Protocol 2 = 500 x 0.5 = 250; Protocol 3 = 100; Protocol 5 = 7500 x 0.6 = 4500; Protocol 6 = 6000 x 0.2 = 1200; Protocol 7 = 1200 x 0.8 = 960; Protocol 8 = 3200 x 0.4 = 1280. Total = 8290 = 17.3%

Severe: Protocol 5 = 7500 x 0.015 = 113; Protocol 7 = 1200 x 0.2 = 240; Protocol 8 = 3200 x 0.6 = 1920. Total = 2273 = 4.7%

What will happen to animals used in this project?

- Killed
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse
- Used in other projects

A retrospective assessment of these predicted harms will be due by 9 January 2032

The PPL holder will be required to disclose:

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

Replacement

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

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

We use animals in our research because there is currently no reliable alternative. Experiments in test tubes or dishes cannot fully copy what happens inside a living body. The heart and blood vessels do not work alone ; they are closely connected with other parts of the body, like the immune system, hormones, and metabolism. For example, after a heart attack, the body responds in many ways at once, involving the heart, brain, blood, and other organs. This can lead to heart failure or weight loss, but these complex changes cannot be reproduced outside a living organism. We also cannot predict in a dish when a blood vessel might weaken and burst. Because of this, we need to study these diseases in living animals to understand how all these systems interact and to safely test whether new treatments could work before they are used in people.

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

Over the last few years, we have been developing ways to study disease using human cells in a laboratory dish, rather than in living animals. In particular, we use a special type of human cell (called pluripotent stem cell) that can be grown in the lab and turned into different cell types, such as the cells that make up artery walls or the immune cells involved in disease. By studying these cells in a dish, we can reproduce some of the same processes that happen inside a living body. This has already allowed us to reduce the number of animals we use in certain experiments. For example, we use specially grown human cells in a dish to study how genetic differences affect the way artery wall cells behave and function. We will keep exploring other non-animal approaches, with the goal of replacing the use of animals wherever possible.

We also use computer programs (a type of “artificial intelligence”) to learn from large amounts of data and predict how diseases develop and how cells behave. These computer models can help us test ideas and narrow down which experiments are most important before using animals.

We estimate that the use of this non-animal method has replaced at least three substantial animal experiments per year, equating to approximately 150 mice annually that would otherwise have been required.

Why were they not suitable?

Other alternatives are simply not good enough to replace animals in every situation. No laboratory dish experiment can accurately predict whether heart failure will develop after a heart attack, why heart

failure leads to weight loss, or when a major artery might burst. For these questions, living animals remain essential.

A retrospective assessment of replacement will be due by 9 January 2032

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 been using mice in our cardiovascular research for 30 years. When planning our experiments, we use statistics and work with expert statisticians to make sure we are using the right number of animals, not too many, but enough to get reliable results. We therefore have a lot of previous data and experience to help us estimate how many animals we will need for this programme of research. This is how we estimated the number of mice needed during the 5 years of the project:

Protocol 6: We plan to use about 6,000 mice over 5 years. For each experiment, we need about 80 mice to compare two groups fairly (40 in each group as per our sample size calculation in Protocol 6). This means we will run around 15–16 experiments each year across several research projects. That is about 3 experiments per year for each project. This number reflects the amount of work we are doing across different studies.

Protocol 7: We plan to use 1,200 mice. Each experiment needs about 60 mice (as per our sample size calculation in Protocol 7), so this allows for about 4 experiments per year in one project.

Protocol 8: We plan to use 3,200 mice. Each experiment needs about 80 mice (as per our sample size calculation in Protocol 8), which means about 8 experiments per year across two projects (around 4 experiments per project).

Protocols 4 and 5 (breeding mice): We plan to use 36,500 mice to breed enough animals for the experiments above (which need about 10,400 mice in total). We need this larger number because not all mice born can be used. For example, some mice do not have the right genes, some experiments require only males, and some special types of mice are harder to breed. These estimates are based on what we have learned from our work over the past 5 years.

The total number of mice for this project may seem high. However, our laboratory is relatively large, with between 15 and 18 researchers, both junior and senior, supported by multiple funded projects and

all carrying out experiments that involve animals.

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

We follow established international guidelines when planning and carrying out our animal experiments, including the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines and recommendations from CAMARADES/NC3Rs (organisations dedicated to improving the way animal research is designed and reported): (https://norecopa.no/prepare, https://www.nc3rs.org.uk/experimental-design-assistant-eda; https://nc3rs.org.uk/3rs-resource-library/3rs-advice-project-licence-applicants#:~:text=3Rs%20advice%20for%20project%20licence%20applicants%201%20Introduction,studies.%20...%203%20Reduction%20...%204%20Refinement%20).

Every experiment follows a carefully planned and tested protocol that is agreed upon before the experiment begins. We clearly define what the main result we are looking for will be (known as the primary endpoint) and make sure the experiment is designed specifically to detect it. We also identify any other factors that could affect this main result, and take steps to make sure these are either controlled for or spread equally across all the groups being studied. To make our results as reliable and reproducible as possible, we use randomisation (meaning animals are assigned to groups by chance) and blinding when possible (meaning the people measuring the results do not know which group each animal belongs). When designing each experiment, we carry out statistical calculations to make sure we are using the smallest number of mice possible while still getting meaningful and reliable results.

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

We follow established guidelines for breeding and colony management (https ://nc3rs.org.uk/3rs-resources/breeding-and-colony-management). We carefully plan our breeding programmes to produce the right number of animals with the correct genetic make-up; for example, to make sure that the gene or genes we have modified are working as intended.

When we have little information to go on, we first carry out small pilot studies to help us better estimate how many animals we will need to get meaningful results.

We always aim to get as much information as possible from each individual animal. For example, rather than using different animals at different points in time, the same animal may be scanned repeatedly using safe, non-harmful imaging throughout the experiment to track how its disease is developing. When animals are humanely killed at the end of a study, we collect tissue samples from multiple organs. This allows us to study the effects of genetic changes across different parts of the body, making the most of every animal used.

A retrospective assessment of reduction will be due by 9 January 2032

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 use well-established and widely accepted methods to study heart and blood vessel diseases. These include experiments in living animals that reproduce the same disease processes seen in patients, such as the build-up of fatty deposits in arteries, heart attacks, heart failure, infection-like conditions following a heart attack, and the bulging or rupture of arteries. We use mice because they have been shown to be very well suited to studying these conditions. Our expertise in these procedures is recognised internationally. We follow the most up-to-date guidelines for animal research, and all animals are housed in appropriate, comfortable, and enriched environments.

By carrying out pilot studies and drawing on more than 30 years of experience with well-established procedures, we keep unexpected effects on the mice to a minimum, and reduce pain, distress, and suffering as much as possible.

In some experiments, animals may experience significant discomfort. This is unavoidable when studying diseases that are themselves very serious, such as severe heart failure with major weight loss, infection-like conditions, or the rupture of a blood vessel. These same conditions cause significant suffering in patients, which is precisely why we are studying them, to find better treatments. In some procedures, the severity of side effects may vary depending on the strain, age, or sex of the mouse. We will make adjustments as the study progresses to ensure that suffering is kept to a minimum at all times. Animals are monitored very closely so we can spot early signs of pain or distress and act quickly, using careful observation and gentle scans when needed, to end the experiment humanely before suffering becomes too great, even though these signs do not always clearly predict serious problems.

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

In the past, we worked with other researchers to explore whether zebrafish could be used to study the build-up of fatty deposits in arteries. Unfortunately, zebrafish do not develop the more advanced stages of this disease, in particular the stages that are responsible for the most serious harm and sometimes death in patients. They are therefore not suitable for this type of research. The same applies to more immature life stages of mice.

In a small number of experiments, procedures will be carried out while the animal is under a full general anaesthetic, meaning it will be completely unconscious and will not feel anything, in order to

study certain aspects of the disease process. Terminal anaesthesia (meaning the animal will not recover) will be considered when very short-term physiological or biological responses are measured.

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

We limit the number of techniques and surgical procedures in each experiment to reduce the impact on each animal while still obtaining reliable scientific results.

We check on our animals very frequently to spot any early signs of pain or distress.

Where needed, we help animals adjust to changes in their environment. For example, when mice arrive from an outside supplier, we allow them a settling-in period to get used to their new surroundings. When we need to measure how much individual animals eat and how their bodies use food, they are housed alone in special cages for up to 10 days. Being housed alone can cause some stress, and some mice may behave aggressively when put back with their cage-mates. To reduce this, we expose them to familiar bedding smells for several days before bringing them back together. When giving injections, we use single-use needles of the right size, and we gradually get the mice used to being handled and held before the day of the injection. When using tamoxifen, we follow established guidance on tamoxifen use. These approaches are in line with expert recommendations on reducing stress in mice (https://www.nc3rs.org.uk/sites/default/files/documents/NC3RsarticleJaneHurst%20making%20sense%20of%20scents.pdf).

We always aim to get as much information as possible from each individual animal. For example, the same mouse may be scanned repeatedly using safe, non-harmful imaging throughout an experiment to track disease progression, rather than using different animals at each time point. When animals are humanely killed at the end of a study, we collect tissue samples from multiple organs to study the effects of genetic changes across the whole body.

We have also developed and improved our surgical procedures for studying aneurysms (the dangerous bulging of blood vessel walls). We now use a simpler surgical technique that causes less damage to the vessel and takes much less time to perform. We will continue to look for ways to improve our methods and reduce animal distress and suffering as much as possible.

We provide supportive care for all animals, including extra nesting material, additional food such as soft wet food, and easy access to food and water, for example by lowering food and water dispensers or placing food directly on the cage floor. We use established, validated scoring systems like grimace scales to monitor animal wellbeing and to decide when pain relief is needed.

Animals are monitored very closely so we can spot early signs of pain or distress and act quickly, using careful observation and gentle scans when needed, to end the experiment humanely before suffering becomes too great, even though these signs do not always clearly predict serious problems. In about 30% of cases, this allows us to end the experiment earlier and humanely, so that the animals do not die suddenly.

We also give pain-relieving medicines whenever they are needed, and before procedures that are expected to cause pain, such as surgery, to reduce pain and make the animals as comfortable as

possible.

As a result of our refinement procedures, the most severe procedures were required much less frequently than initially anticipated. Although severe severity was permitted in the previous project licence, we reduced the number of animals experiencing severe effects by approximately 40–50% compared with our original estimates. This improvement was possible because we have gained more experience using these models and because the animals are monitored very closely, allowing us to identify problems earlier and take action sooner.

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

We use well-established experimental procedures and follow a range of accepted international guidelines (ARRIVE 2020, 2nd edition,https://arriveguidelines.org/) to make sure our animal experiments are carefully planned, properly reported, and carried out to the highest possible standard, with animal welfare always in mind. These guidelines cover all aspects of our work, including surgical procedures, and are produced by leading organisations dedicated to improving the way animal research is conducted (https://www.lasa.co.uk/current_publications/), Norecopa guidelines (https://norecopa.no/3r-guide/guiding-principles-for-preparing-for-and-undertaking-aseptic-surgery).

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

We are at the forefront of cardiovascular research and are committed to continuously improving the way we carry out animal studies. We stay up to date with the latest developments in the 3Rs: the principles of Replacing, Reducing, and Refining the use of animals in research (https://nc3rs.org.uk/3rs-advice-project-licence-applicants-refinement). We actively follow leading organisations and resources dedicated to the 3Rs, including Norecopa platform (https://norecopa.no/alternatives/the-three-rs), ATLA (Alternatives to Laboratory Animals) Journal: https://journals.sagepub.com/home/atla), LASA (https://www.lasa.co.uk/current_publications/) and the Danish 3R-Centre (https://en.3rcenter.dk/), and aim to put any new recommendations into practice as quickly as possible.

The Named Information Officer at our establishment are also an invaluable resource of 3Rs information.

A retrospective assessment of refinement will be due by 9 January 2032

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?