Home Office

NON-TECHNICAL SUMMARY

Early life origins of heart disease in mammals

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

Key words

fetus, cardiovascular, heart, circulation, maternal

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

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

To determine some of the mechanisms via which adverse conditions during pregnancy or early postnatal life trigger an increased risk of heart disease in mammals

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 disease is one of the greatest killers in the world, causing one in four deaths in the UK today. Therefore, understanding mechanisms that contribute to the risk of cardiovascular disease is important to design interventional therapies. While our genetic makeup interacts with lifestyle factors, such as smoking, an unhealthy diet, or a sedentary life to influence the risk of heart disease, strong evidence suggests that adverse conditions during pregnancy or early postnatal life are just as, if not more important in determining cardiovascular risk in the adult offspring. This concept of the 'Developmental Origins of Health and Disease' has brought attention to the study of complicated pregnancy, and to consider intervention strategies to reduce the cardiovascular burden as early as possible during development, even in pregnancy. Thus, it is important to undertake this work to understand how suboptimal conditions during development may influence the cardiovascular risk in progeny. Understanding mechanisms will shed light into designing interventional therapies. The data generated has therefore the potential to hasten translation to rational interventions not only to treat mother but also her offspring. This will help reduce the vast burden of pregnancy-induced origins of cardiovascular disease, thereby having a major clinical, economic, and societal impact on cardiovascular health.

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

The outputs that we will see at the end of the project will be:

1. Data relating to cardiovascular function, cardiovascular morphology (structure) and underlying molecular pathways in pregnant animals and their fetal, newborn or juvenile/adult offspring exposed to control or adverse pregnancy (e.g. lower than normal oxygenation or hypoxia, obesogenic diet, altered ambient temperature) with and without treatment (e.g. antioxidants). Data relating to cardiovascular function will include measurements of arterial blood pressure, heart and blood flow measured in circulations of interest during basal and stimulated conditions. Data relating to changes in cardiovascular structure may include alterations in the thickness of the walls in the heart chambers and

in different vessels (e.g. aorta). Data relating to molecular pathways will include the measurement of signalling mechanisms at the level of the gene and the expression of important proteins.

2. Data relating to cardiovascular function, cardiovascular morphology (structure) and underlying molecular pathways in first and second generation animals raised from control or adverse pregnancy (e.g. lower than normal oxygenation or hypoxia, obesogenic diet, altered ambient temperature) with and without treatment (e.g. antioxidants);

3. Data relating to cardiovascular function, cardiovascular morphology (structure) and underlying molecular pathways in post-partum females exposed to control or adverse pregnancy (e.g. lower than normal oxygenation or hypoxia, obesogenic diet, altered ambient temperature) with and without treatment (e.g. antioxidants);

4. Publications as abstract, papers, reviews and book chapters of Points 1-3;

5. Dissemination of unsuccessful approaches or findings.

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

In the short-term term (1-3 years), the data outputs of this project licence (e.g. changes in arterial blood pressure, heart rate, cardiac function) will inform us how adverse conditions during early life may increase the risk of cardiovascular dysfunction later on in the adult offspring, as well as in the pregnant and post-partum mother. For instance, is the heart affected more than the vasculature? Which is worse and which may contribute more to triggering cardiovascular disease?

In the medium-term (3-5 years), the data outputs of this project licence will tell us which are the areas to focus on, and what similarities and differences there are between species. The idea is then to create a layered approach of understanding on the effects of adverse conditions during development on the cardiovascular system across the life course in different species. The work will generate collaborations seeking to address questions with diferent expertise, usually involving work ex vivo in tissues generated from this project. Therefore, other scientists will also benefit from the work.

Combined, all outputs will help to better translate our findings to the human clinical situation. For example, knowledge of common effects of an adverse condition in early life on the heart of the mouse, rat and sheep may precipitate human benefit by designing a treatment with protection across species and thereby likely to be as efficient in humans.

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

The outputs of this work, including the dissemination of unsuccessful approaches or findings, will be maximised at several levels:

Scientific advancement and collaboration: In the longer term (>4 years), the data will benefit the design of therapies in higher vertebrate models of adverse pregnancy with a view to human translation and the design of clinical trials. This will be achieved via collaboration with experts in different fields. Therefore, the proposed work in this new project licence may hasten translation to relatively simple but novel human clinical interventions to not only treat the mother, but also her progeny. This will contribute

to a reduction in the burden of developmental origins of heart disease, thereby having a positive clinical, economic and societal impact on health.

Dissemination of new knowledge. Other pathways to further increase impact will include contacting the funders and the institution's communications office to alert them of the potential influence for human health of the scientific findings. This will lead to press releases, which will be supported by radio and television interviews. In addition, the data will benefit the design of cures to protect the health of the unborn child. The proposed research is therefore likely to be of significant interest and benefit not only to researchers carrying out similar or related research in the field, but also to national and international researchers in other disciplines, such as biochemistry, pharmacology and nanotechnology, as well as cross-disciplinary teams in the pharmaceutical industry. To deliver translational benefit to the nation’s health, wealth and culture we will adopt a number of strategies such as seeking patent protection for any new therapies or diagnostic biomarkers revealed by the research as well as actively engage with the commercial pharmaceutical and healthcare sectors to exploit our research at the earliest opportunity.

Species and numbers of animals expected to be used

- Mice: 4000
- Rats: 4000
- Sheep: 3500

Predicted harms

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

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

Types of animals

Working with different mammalian species provides an integrative approach to understanding the problem using each animal species for its own particular strength.

The work with sheep has several advantages. First, the cardiovascular development is similar between sheep and humans, enhancing the clinical translation of the work. Secondly, the sheep is the only established animal model in which the mother and the fetus can be surgically prepared under general anaesthesia with catheters, probes and electrodes to record alterations in physiology in long-term preparations following full post-surgical and anaesthetic recovery. Similar insight does not exist for any other species.

Similarly, the work with rats and mice has several advantages. First, both rats and mice are litter-bearing, meaning that different pups from the same litter can be used for different outcome variables, reducing the number of pregnancies needed (1 pup for studies in vivo (in the living organism), 1 pup for studies ex vivo (after death) in isolated organs, such as the heart preparation, 1 pup for fixation for subsequent histology (using a microscope) studies, 1 pup for freezing of isolated organs for

subsequent molecular studies). Secondly, litter-bearing animals means that we can compare outcomes in male and female pups from the same litter, again reducing the number of pregnancies needed. Studies controlling for the effects of sex of the offspring on outcome variables are highly encouraged by the Home Office and grant-awarding bodies. Thirdly, rats and mice have shorter lifespans, facilitating studies across generations in a reasonable time-frame, for example during the tenure of a Project Licence or a PhD.

Work with rats offer an important advantage over mice. Their larger size means that more detailed experiments can be done in the adult offspring in the living animal. This includes, for instance, cardiovascular recording during basal conditions and in response to treatment, e.g. drugs administered. In turn, work with mice offer some important advantage over rats. Previous work in our laboratories and by others have used mice preferentially rather than rats for several studies of adverse pregnancy, for instance studies of maternal obesity during pregnancy. Using mice for some studies in this project licence means that we can compare data generated to a much greater body of existing genetic tools and literature.

Choice of life stages

The studies focus on pregnancy, fetal, newborn and adult offspring as the project is designed to determine the effects of adverse conditions during pregnancy on the cardiovascular system of progeny across the life-course, from the fetal stage through to the adult offspring. The studies also focus on the pregnant mother and the post-partum mother, as there is evidence that adverse pregnancy conditions, such as obesity, can also increase the risk of heart disease in the mother not only during, but also long after pregnancy.

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

The studies focus on pregnancy, fetal, newborn and adult periods. This is because the project is designed to determine the effects of adverse conditions before and during pregnancy on the cardiovascular system of progeny across the life-course, from the fetal stage through to the adult offspring. The studies also focus on the mother during pregnancy and after birth. This is because evidence suggests that adverse conditions during pregnancy can also affect the maternal cardiovascular health during pregnancy and long after birth.

To study the mother during pregnancy or in the post-partum period, or to study the offspring in the fetal, newborn or juvenile/adult period, typically a pregnancy will be exposed to control (e.g. normal air or normoxia with or without a treatment, such as antioxidants, or glucocorticoids or stem cells or miRNAs) or challenged conditions (e.g. lower than normal oxygenation or hypoxia with or without a treatment, such as antioxidants, or glucocorticoids or stem cells or miRNAs) during the whole or part of the pregnancy (e.g. the last third of gestation). Alternatively, a pregnancy may be exposed to control ambient temperature (with or without a treatment, such as antioxidants, or glucocorticoids or stem cells or miRNAs) or challenged conditions (high ambient tempreature with or without a treatment, such as antioxidants, or glucocorticoids or stem cells or miRNAs) during the whole or part of the pregnancy (e.g. the last third of gestation). In all cases above, the majority of the studies will be ex vivo (after death), i.e. in tissues isolated from animals following Schedule 1 killing. A minority of studies will investigate the function of the heart and circulation under terminal or recoverable anaesthesia following surgery (e.g studies using sheep). Under terminal or recoverable anaesthesia, an animal at the

appropriate stage of the lifecourse (e.g. mother during pregnancy or in the post-partum period, or offspring in the fetal (sheep only), newborn (sheep only) or juvenile/adult period) will be surgically prepared with catheters, electrodes and probes to record cardiovascular function in the living organism. Under terminal anaesthesia, typically 1 experiment will be performed in any one animal, lasting approximately 5 hours. For animals undergoing recoverable anaesthesia procedures, there will be typically 1 exposure to recoverable anaesthesia. Then, following 1-5 days of post-surgical recovery, typically there will be 1 experiment performed on any one day, lasting approximately 5 hours. Typically, there will be 4 of these daily experiments following exposure to one recoverable anaesthetic, typically with 1-2 rest days in between experimental days.

Similarly, to study the effect of obesity during pregnancy on the maternal and offspring physiology, non pregnant animmals will be fed a control or an obesogenic diet (typically for 2-3 months in sheep or 3-6 months in rodents) prior to conception. Control or obese pregnancies may then be treated (e.g. typically in the last third of gestation with vehicle or antioxidants, or glucocorticoids or stem cells or miRNAs). As above, the majority of the studies in the mother, fetus or adult offspring will be ex vivo (after death), i.e. in tissues isolated from animals following Schedule 1 killing. A minority of studies will investigate the function of the heart and circulation under terminal or recoverable anaesthesia following surgery (e.g studies using sheep). Under terminal or recoverable anaesthesia, an animal at the appropriate stage of the lifecourse (e.g. mother during pregnancy or in the post-partum period, or offspring in the fetal (sheep only), newborn (sheep only) or juvenile/adult period) will be surgically prepared with catheters, electrodes and probes to record cardiovascular function in the living organism. Under terminal anaesthesia, typically 1 experiment will be performed in any one animal, lasting approximately 5 hours. For animals undergoing recoverable anaesthesia procedures, there will be typically 1 exposure to recoverable anaesthesia. Then, following 1-5 days of post-surgical recovery, typically there will be 1 experiment performed on any one day, lasting approximately 5 hours. Typically, there will be 4 of these daily experiments following exposure to one recoverable anaesthetic, typically with 1-2 rest days in between experimental days.

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

It is well established that pregnancy complicated by adverse developmental conditions (e.g. lower than normal oxygenation, or maternal obesity, or exposure to glucocorticoids, or exposure to high ambient temperature) can reduce fetal growth and survival. Our own experience is that relative to controls, adverse pregnancy can reduce fetal growth by 25-30% in 80% of fetuses. It is also well established that relative to controls, fetuses from adverse pregnancies are much more vulnerable to acute challenges in late gestation, such as compression of the umbilical cord modelling prolonged labour. Clinically, the reason for this is completely unknown and trying to find out is one of the main points of this project. As it happens clinically in humans, we expect that a large proportion of fetuses from adverse pregnancy (80%) will not survive an acute challenge (e.g. umbilical cord compression) in utero. If fetuses from adverse pregnancy are allowed to deliver naturally, we expect some of them not to survive the birth process (20%). Relative to newborns from control pregnancies, surviving newborns of adverse pregnancy show a ca. 25% reduction in body weight and reduced growth rates until adulthood. Relative to offspring from control pregnancies, we expect that those from adverse pregnancy will be more vulnerable to acute challenges in the juvenile or adult period, and a minority of them (10%) may not survive experimentation in adulthood. Why adult offspring from adverse pregnancy are at increased risk during acute challenges is again one of the main questions that this project is aiming to address.

Administration of substances in sheep can result in (on occasion) preterm delivery/abortion. Therefore, we expect that administration of some substances (e.g. glucocorticoids, antioxidants, stem cells, miRNAs or vehicle) during pregnancy in all species studied may similarly induce preterm delivery/abortion in 10% of animals over the life of the project licence. It is also possible that administration of substances may induce inflammation, swelling and infection in 10% of animals studies at any stage of the lifecourse, over the life of the project licence.

While under terminal anaesthesia, some protocols may require surgical implantation of probes or occluders, or the application of vessel occlusion. Some of these procedures may lead to unexpected bleeding. If more than 10% of the estimated blood volume of the animal is lost, then the animal will be killed by a Schedule 1 method.

A minority of experiments in the newborn, juvenile or adult offspring require surgery under recoverable anaesthesia. All surgical procedures will be carried according to the Home Office Minimum Standards for Aseptic Surgery. Through previous work, we have gained significant experience with experimentation on rats and sheep following surgery. Pain killers will be administered as required, judging from the animal behaviour. In some animals, arterial catheters will be placed which allow for blood sampling. The health of the animal can then be monitored through measurements of blood gases. For example, we will be able to ask if oxygen levels in blood are normal? We expect most of the animals to survive, but there is an increased risk of death (20% over the life of the project licence) during or following surgery under any type of anaesthesia. In preparations, where the fetus is surgically prepared with significant instrumentation (e.g. catheters, probes, eletrodes, occluders) there is a greater chance of accidental occlusion of the umbilical cord and fetal death (20% over the life of the project licence). Pregnant animals undergoing recoverable anaesthesia can also present with abdominal hernias (10% over the life of the project licence). To minimise this possibility, animals are dressed in tubigrip around their abdomen to increase support.

Some protocols require study of the animal after nerve transection or removal of an endocrine (hormonal) organ (e.g. removal of the nerve that is attached to the carotid body or removal of the adrenal glands). Denervation of the carotid bodies does not produce any resultant harm. In fact, carotid body denervation is currently being trialled in human patients to treat hypertension. In contrast, removal of the adrenal glands can trigger adverse effects on blood volume and blood pressure over prolonged periods of time. Adrenal insufficiency in man and other animals is known as Addison’s disease and these individuals through hormone insufficiency can develop low blood pressure over a period of months. To minimise this possibility, no post-natal animal will be studied and kept for longer than 1 month after surgical removal of the adrenal glands.

In the event of post-operative complications, such as a catheter being removed, animals will be killed unless such complications can be remedied promptly and successfully using no more than minor interventions. In the case of wound dehiscence (bursting open), uninfected wounds may be re-closed on one occasion.

We estimate that most animals will recover well from surgery under recoverable anaesthesia. However, we expect that 10% of animals will not recover well. In all cases, the animal's food intake and ability to pass faeces will be closely monitored at least twice daily. Following NVS advice, any animal that does not show appropriate food intake, defecation, blood gases and blood glucose concentration for >72h will be killed by a Schedule 1 method or earlier if its condition deteriorates before this point.

For studies involving a scan of the heart in the conscious animal, restraint of the animal is necessary. Usually, we use the same person for restraining any one animal, so the animal gets used to the environment, and we minimise the duration of restraint, typically 30 minutes. Combined, these strategies minimise the animal's discomfort.

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)?

Species: Sheep

Mild: 80%

Moderate: 20%

Species: Rat

Mild: 90%

Moderate: 10%

Species: Mice

Mild: 90%

Moderate: 10%

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

- Killed

Replacement

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

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

In some experiments, we must use whole animals or organs isolated from animals because function, for instance in the cardiovascular system, is regulated by complex networks, none of which have been reconstituted completely in computer models. The overall system is not well enough understood to make mathematical modelling useful.

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

There are no suitable non-animal alternatives to use in this project for the majority of the work. A small component of the work could be achieved by investigating isolated organs and tissues after death, from non-regulated investigation in PM tissue/organs. It may be possible in future to extend some of the work by using cell lines to identify possible signalling pathways and to test candidate therapies.

Why were they not suitable?

For the majority of the work, no non-animal alternatives are suitable to use in this project as none can model the effects of adverse conditions during embryonic/fetal development in programming an increased risk of cardiovascular disease in the adult progeny, or in the post-partum mother.

Reduction

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

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

We have estimated the number of animals that we will use in this licence from experienced use of animals, as detailed in more than one retrospective review using these species.

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

The experimental design was created with the NC3R's Experimental Design Assistant to ensure reproducibility using the least number of animals to satisfy statistical power for anyone measurable output.

To ensure the minimum number of animals is used in this project to address all objectives, we have considered the choice of species very carefully. Sheep are used in some experiments because it is the only established animal model in which the mother and the fetus can both be surgically prepared under general anaesthesia with catheters, probes and electrodes to record alterations in physiology in long-term preparations following full post-surgical and anaesthetic recovery. Similar insight does not exist for any other species. However, we will reduce the number of experiments where surgically prepared animal preparations are required, and the majority of the work will be done in tissues isolated from animals after death. A large component of the work will be done using rats and mice. These species are litter-bearing, meaning that different pups from the same litter can be used for different outcome variables, reducing the number of pregnancies needed (1 pup for studies in vivo (in the living organism), 1 pup for studies ex vivo (after death) in isolated organs, such as the heart preparation, 1 pup for fixation for subsequent histology (using a microscope) studies, 1 pup for freezing of isolated organs for subsequent molecular studies). Secondly, litter-bearing animals means that we can compare outcomes in male and female pups from the same litter, again reducing the number of

pregnancies needed. Studies controlling for the effects of sex of the offspring on outcome variables are highly encouraged by the Home Office and grant-awarding bodies. Therefore, this significantly contributes to the 3Rs principle of reduction as enshrined in EU Directive 2010/63.

Where relevant, multiple experimental designs will be used, rather than the one-thing-at-a-time approach, to maximise the information obtained from the minimum resource. Therefore, in the same animal, there may be studies in the living organism as well as in isolated organs after death. For most experiments, the study design will adopt methods of analysis previously published extensively by our group, which compares 4 groups: control and experimental groups with and without a treatment or intervention. For example, outcomes from normoxic (normal air) or hypoxic (lower than normal oxygenation) pregnancies with and without treatment with an antioxidant. Control groups treated and untreated are necessary, as the treatment may affect normal and complicated pregnancies differentially.

Sex differences are an important consideration in the risk of developing cardiovascular disease. Therefore, assuming a 1:1 ratio of males to females, the number of animals required per outcome variable will be at least doubled to be able to address sex differences. In such cases, statistical analysis able to compare three factors comparing treatment, intervention and sex will be adopted (e.g. a Generalised Mixed Linear Model; SPSS).

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 optimise the number of animals used in this project from 1) pilot data and 2) by using multiple data obtained from the same animal. For instance, we will obtain data from the living organism as well as from tissues isolated from them after death. In addition, we routinely share tissues generated from projects for collaborative studies by other investigators. About 20% of our publication output is derived from such collaborative studies.

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 project has been designed such that the majority of the work can be achieved by investigating isolated organs and tissues. For example, after death, the function of the isolated heart and vessels can still be investigated, as well as experiments at the level of the cell and molecule. Comparatively smaller components of the work will involve studying whole living animals under terminal anaesthesia, or conscious animals which have been surgically prepared under general anaesthesia. In some cases, it is necessary to study conscious animals, as anaesthesia can impair normal cardiovascular function.

Experiments will only be performed following appropriate post-surgical recovery. We will keep suffering to the minimum by using procedures with the least possible severity, and by subsequent monitoring with veterinary advice.

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

Other projects under licence are studying the effects of adverse developmental conditions in less sentient species, using birds, to isolate the effects of challenges on the embryo independent of effects on the mother and/or placenta. However, to increase the clinical translation of the work, one has to extend those studies using mammals, which is the purpose of this project.

The development of the cardiovascular system in other less sentient species, for instance reptiles, amphibians, worms or flies is very different than in humans, as these species are ectothermic (creatures that must rely on the temperature of their physical environment to regulate their internal body temperature), in addition to not having a placenta. The regulatory mechanisms of cardiovascular function in such species are not well understood.

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

With experience from previous work, we have streamlined and refined surgical procedures that reduce bleeding, shorten anaesthetic exposure and improve port-surgical recovery. For example, most surgeries under recoverable anaesthesia are completed within 2.5 hours. We have found that exposure to recoverable anaesthesia for <3h significantly accelerates post-surgical recovery minimising harms.

We will refine protocols by ensuring that all surgical procedures are carried at least to the Home Office Minimum Standards for Aseptic Surgery. In addition, any animal exposed to surgery under recoverable anaesthesia will be observed and scored for any signs of pain, administered pain killers and antibiotics, appropriate for the species and life-stage, to minimise discomfort and infection. We will explore palatable substances for voluntary pain treatment rather than injection such as flavoured paste or apples (sheep). This will minimise possible resultant harms and maximise physiological outcome.

In some animals, arterial catheters will be placed which allow for blood sampling. The health of the animal can then be monitored through measurement of blood gases and acid/base status on a daily basis, maximising monitoring, post-operative care and pain management.

The animal's appetite and general demeanour will be noted. The rectal temperature will be taken to determine changes in body core temperature. Alternative food may be provided (e.g. offering grass to sheep to encourage eating). The animals wil, be weighed routinely to ensure appropriate body weight is maintained.

Further refinement such as the use of group housing, sedation, acclimatisation to handling and environmental enrichment will be implemented to ensure the animals are less stressed and well cared for. The animal's quality of bedding will be reviewed routinely. We will continue our practice to consult with the NACWO to balance the harms and improve the welfare of the species we are using.

A component of the work involves protocols in sheep. Whenver possible, sheep will be housed as flocks in barns and paddocks with access to hay and grass. The duration of time that sheep will spend indoors in a laboratory environment is always minimised.

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

To ensure experiments are conducted in the most refined way, we follow the NC3Rs' ARRIVE guidelines, the LASA guidelines and the PREPARE guidelines.

For example, we will refer to the latest edition of the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery 2017, at the time of preparing this project licence application.

The Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines issued by NORECOPA (https://norecopa.no/prepare) covers all stages of quality assurance, from the management of an animal facility or population to the individual procedures which form part of a study.

We will refer to specific guidance or position papers from the Laboratory Animal Science Association, (LASA) https://www.lasa.co.uk/current_publications/. For example: Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T (2018) PREPARE: guidelines for planning animal research and testing. Lab Animal 52(2): 135-141. doi: 10.1177/0023677217724823.

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

Project licence holders are ultimately responsible for implementing the 3Rs within their work. Therefore, the project licence holder will have regular discussions with the Named Persons and animal technicians to review current approaches and whether there are any new 3Rs opportunities.

We will use of other resources, such as Norecopa https://norecopa.no/databases-guidelines. Regular consideration and reflection of the latest practical guidance from Laboratory Animal Science Association (LASA) will provide additional sources of new recommendations and advances in animal techniques.