Home Office

NON-TECHNICAL SUMMARY

Deciphering the mechanisms of heart failure and arrhythmias

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

heart failure, atrial fibrillation, myocardial infarction, arrhythmia, therapy

Animal types	Life stages
Sheep	Adult, Aged animal

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?

We aim to identify what is responsible for the impaired ability of the heart to pump blood and changes to the structure and electrical properties of the heart leading to abnormal heart rhythms in common cardiovascular diseases such as heart failure, atrial fibrillation and after a heart attack. We will then determine the effectiveness of new treatments in reversing these changes and restoring the pumping function of the heart and restores the hearts normal structure and electrical properties.

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?

Diseases of the cardiovascular system remain the leading cause of death. Heart failure is a condition where the heart is unable to pump blood properly and affects approximately 1 million people in The UK. The causes of heart failure are varied and it can develop following a heart attack, infection, through genetic changes or as a consequence of having high blood pressure, being overweight and old age. Heart failure patients have worse survival outcomes than those with the common cancers such as breast or prostate cancer.

In certain types of heart failure, notably those which are caused by abnormally high heart rates, we know that correcting the abnormally high rate can result in recovery from heart failure. However, in some patients the abnormally high heart rate is only temporarily corrected by either surgical, electrical or drug based treatments and in these patients heart failure can recur. In clinical practice, identifying which patients are most at risk of recurrence remains a major unmet need.

In addition to heart failure where the heart muscle fails to either contract or relax properly, many diseases of the heart also cause abnormalities in the rhythm of the heart. One form of abnormal heart rhythm known as atrial fibrillation is particularly common, with 1 in 3 people over the age of 45 years having a lifetime risk of developing atrial fibrillation. Whilst atrial fibrillation may not itself be immediately life threatening, it does markedly increase the risk of stroke and developing heart failure.

Abnormal heart rhythms can also occur as a consequence of taking certain drugs that are commonly used including antidepressants, drugs to treat fungal infections and drugs used to treat certain types of cancer. A major problem clinically is that it is difficult to identify which patients receiving these drugs will go on to develop life threatening abnormal heart rhythms.

This programme of work will use models of heart disease that closely resemble the clinical conditions of heart failure, heart attack, atrial fibrillation and drug-induced abnormal heart rhythms. These animal models will allow us to better understand the mechanisms that give rise to heart failure and identify and evaluate new therapies to treat the effects of heart failure and hopefully reverse the changes that occur

in the heart in heart failure. We will also understand why some patients develop life threatening abnormal heart rhythms when taking certain drugs.

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

The programme of work will generate new information on the changes that occur in the heart during the development of heart failure and the mechanisms that bring about abnormal heart rhythms. We will also understand the mechanisms of action of new therapies that are designed to reverse the changes that occur in heart failure and prevent abnormal heart rhythms.

This information will be published as research articles and reviews in open access journals so that it is available to everyone. We will also submit the underlying data in these publications and associated gene and protein datasets to open access databases so that it can be reused by others.

We will present our work to scientific conferences, other academic institutions and public engagement events and through appropriate media channels to maximise dissemination of information and adoption of findings.

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

The immediate term beneficiaries will be scientists who are engaged in either understanding the fundamental biology of this system, or turning scientific discoveries made in the lab into treatments or tools.

In the medium term, we would expect the enhanced mechanistic understanding, target identification and evaluation of therapies to be of benefit to the pharmaceutical industry and device companies pursuing novel therapies to tackle cardiovascular disease.

In the longer term we expect the proof of concept target validation studies in this programme of work to support target therapy progress along the clinical trial pipeline and ultimately the implementation of new therapies for cardiovascular disease leading to patient benefit.

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

We will use the following approaches to maximise the outputs from this work:

- Publication research findings in open access journals (including negative results)
- Submission of developed datasets and supporting analytical coding to open access repositories
- Publication of detailed protocols for new models / refined approaches and methodologies
- Presentation of data and methodologies to scientific, clinical and patient group conferences, symposia and meetings
- Presentation to academic colleagues through institutional seminar programmes
- Contributing to funder awareness events and publications

- Engagement with relevant media outlets (written, spoken, visual) to maximise dissemination

Where collaboration with external partners in the form or service work is undertaken, this is likely to be performed under confidentiality agreements and will have secure data-handling procedures in place. Here, no identifiable client information, proprietary methods, or sensitive data will be shared externally. Any knowledge shared such as methodological improvements, validation data, or insights into unsuccessful approaches will be fully anonymised, aggregated, and stripped of client-specific context

Species and numbers of animals expected to be used

- Sheep: 1150

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.

A key consideration in this programme of work is that less sentient species including Zebrafish or lower mammals such as mice and rats lack important structures within some cells of the heart known as transverse tubules. These structures are present in human heart cells and therefore it is important that any preclinical model used to investigate cardiac disease possess these same structures. Importantly, sheep cardiac cells also contain transverse tubules making them a highly suitable model for human heart disease modelling.

This project will use adult and aged sheep (human equivalent ~ 60 - 75 years). The sheep heart is comparable in size and anatomy to human and their electrical properties and pumping function are much more comparable to human than rodents or other commonly used small animal species. The electrical and pumping similarities of the sheep heart to human are important for this programme of work where we are trying to understand the basis of cardiac dysfunction and abnormal heart rhythms that occur in people, and in particular identifying why some people are more at risk of adverse events than others. This type of predictive modelling would be much more difficult to achieve in rodents where pumping function and electrical properties are controlled very differently. Additionally, the similarity in size and anatomy to human ensures that surgical techniques and devices used in our studies are directly applicable to future work in patients.

We will be mainly using mature adult animals with a smaller number of aged animals used in some studies where we are particularly interested in understanding the role of age as a cofactor in certain diseases of the heart. The use of mature (adult) animals removes some key confounding considerations on cardiac anatomy and function associated with the impact of rapid growth phases in early life present when porcine models are used. Additionally, the cardiac diseases we are studying are mainly present in the adult and aged population.

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

Animals will normally be housed in groups and have constant access to food and water. Where animals undergo general anaesthesia, they will be singly housed in adjacent pens for the immediate post operative recovery period (typically overnight) before being returned to their group accommodation.

Approximately 35 % (400) of the animals used in this project will not undergo any surgical procedure before being humanely killed. We will then isolate cells from their hearts and use these for *in vitro* experiments performed in the laboratory. These animals will only experience very brief periods of discomfort during placement of the intravenous catheter through which drugs are administered immediately before humane killing.

The majority of the remaining animals (~ 44% of the total number animals used in this program of work across all protocols) will typically undergo one minimally invasive surgical procedure under general anaesthesia where we either: i) place a pacemaker under the skin on the side of the neck through an ~ 4cm skin incision and position a cardiac pacing lead into the heart through a vein in the neck accessed through the same skin incision and/or, ii) through the same sized skin incision, we access the artery in the neck and use this to introduce an inflatable tube in one of the vessels of the heart which we then inflate to induce a heart attack (myocardial infarction). Both of these surgical methods use the same procedures that are used in patients to respectively, implant pacemakers or unblock coronary arteries after a heart attack. For pacemaker implants, the procedure typically lasts 1 - 1.5 hours and for the myocardial infarction procedure, surgery lasts 3 - 4 hours as the tube is inflated for 90 minutes before being removed.

Following full recovery from the surgical procedure, the pacemaker is switched on causing the heart to beat faster and induce either heart failure with impaired contractile function or atrial fibrillation. Animals are monitored closely for the development of clinical signs of heart failure and once these are present this marks the end of the experiment and animals will be killed and their hearts removed from studies performed in the laboratory. Where we are studying myocardial infarction (heart attacks), animals are also monitored for the development of clinical signs of heart failure and are again killed and their hearts used in laboratory experiments. For heart failure induced by rapid pacing of the heart, experiments typically last for ~ 12 weeks and for atrial fibrillation (where the upper parts of the heart have an abnormal rhythm) or myocardial infarction protocols, they may last up to six months. In approximately half of these animals we would also investigate the effectiveness of either drugs or stimulation of the vagus nerve as treatments for heart failure, atrial fibrillation or myocardial infarction. Normally this involves the once daily oral dosing with a tablet for ~ 3 weeks.

In some of the animals where a pacemaker is used to induce heart failure we will either: i) deactivate the pacemaker as soon as signs of heart failure are observed (~ 10 - 15 % of pacemaker induced heart failure animals). This is associated with rapid (hours) resolution of signs of heart failure and allows us to study the processes associated with recovery from heart failure. Here, experiments last ~ 16 weeks in total; Or, ii) deactivate the pacemaker as soon as signs of heart failure are observed (~ 10 - 15 % of pacemaker induced heart failure animals), allow them to recover from heart failure for ~ 5 weeks before restarting the pacemaker to induce heart failure on one additional occasion. This will allow us to study whether the susceptibility to heart failure is altered by prior disease occurrence and if so, whether the mechanisms involved are different. These experiments would be expected to last for ~ 20 weeks in total.

In a further ~ 13% (150) of the total number of animals used in this project, we are investigating a particular type of heart failure associated with impaired relaxation of the heart. These animals will receive a monthly injection of a hormone under the skin in combination with a low concentration of salt in their drinking water to raise blood pressure. We may also give these animals a diet that causes them to gain weight. We will also monitor these animals for development of clinical signs of heart failure and if present they will be killed and tissues harvested for laboratory experiments. These experiments will last for up to six months.

In all animals which receive a pacemaker implant, myocardial infarction or dietary modifications we will also periodically; i) take blood samples from a superficial vein in the neck or leg, ii) measure the ECG using skin electrodes applied to the legs and, iii) measure blood pressure using a blood pressure cuff around the tail. These procedures would be performed no more frequently than once approximately every week and should result in minimal transient discomfort to the animals. For these procedures animals are conscious and gently manually restrained and procedures would not typically be conducted more than once per week.

In some of the heart failure, myocardial infarction or high blood pressure animals we will also conduct additional procedures under general anaesthesia to monitor the electrical properties of the heart. Normally, this will involve: i) placing a catheter through the skin into a vein in the hind leg or side of the neck and introducing a lead into the chambers of the heart to measure the electrical activity of the heart. At the end of the procedure the leads and catheters are removed and pressure applied to the skin puncture site to stop any bleeding in the same way as following blood sampling procedures in man; and/or, ii) under general anaesthesia allowing the animals to breath a reduced oxygen concentration (13 % for ~ 5 minutes) equivalent to that present in high altitude cities in The Andes or Tibet. This allows us to assess key cardiovascular reflexes and has no adverse or lasting effect on the animals. These procedures would not be expected to last more than 1 - 2 hours in total if combined and should not produce more than transient mild discomfort at the site where any intravenous catheter was temporarily placed. Typically animals would not receive more than 2 of these procedures followed by recovery from anaesthesia with at least two weeks between procedures.

The final ~ 13% (150) of animals used in this programme of work will be treated with a commonly used type of drug (such as one of an antidepressant, antifungal or anticancer medication) usually by once daily oral dosing, and we will monitor the electrical activity of the heart using skin ECG electrodes weekly. These drugs will be administered for three weeks and then animals will undergo a procedure to assess the electrical properties of the heart under terminal anaesthesia.

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

For all surgical procedures we would expect animals to make a quick recovery from the procedure and be returned to their housing group the next day. All surgical procedures are performed using minimally invasive approaches through a small skin incision and as such we do not anticipate pain or discomfort from these procedures which cannot be suitably managed with the administration of painkillers at the time of surgery in the same way as patients undergoing the same sorts of procedures.

Where we are studying the effects of heart failure using pacemakers to stimulate the heart, we expect all animals will develop clinical signs of heart failure in approximately 6 weeks which *could* include increased breathing effort, tiredness and coughing. We monitor animals closely for these signs and

once they are present, this marks the end of the experiment for the vast majority of animals with pacemaker induced heart failure (80 - 90% of animals where heart failure is induced) and the animal is humanely killed and tissues harvested for use in laboratory experiments. In the remaining 10 - 15% of animals where heart failure has been induced using a pacemaker, we will allow them to recover from heart failure for a period of time (typically 4 - 6 weeks) before using the pacemaker to re-induce heart failure on one more occasion. This closely replicates the clinical observation where abnormally high heart rates can recur and lead to a second bout of heart failure in patients.

In animals where we are inducing myocardial infarction, during the surgical procedure when the animal is anaesthetised we do expect about half of the animals to develop an irregular heart rhythm and in a small number of these animals this will not respond to drug or device treatment and will lead to death during anaesthesia. We do not expect these animals to experience any pain or discomfort as they are anaesthetised. Following recovery from anaesthesia, animals will be monitored for the development of signs of heart failure and, once observed, this marks the end of the experiment and animals will be humanely killed and tissues used for laboratory experiments.

In animals where we are inducing heart failure due to high blood pressure and obesity, the signs of heart failure would be similar to those above and may take up to 6 months to become evident. However, once signs of heart failure are observed, this marks the end of the experiment and animals will be humanely killed and tissues used for laboratory experiments.

In both the heart failure models, atrial fibrillation and myocardial infarction studies, there is a small possibility that abnormal rhythms in the main chambers of the heart could occur which result in the rapid loss of consciousness and death. If this occurs, we would expect this to happen over a just a few seconds and, due to the rapid loss of consciousness, not result in lasting pain or discomfort.

We will also investigate the impact of drugs or the use of nerve stimulation as potential therapeutic approaches in both models of heart failure, following myocardial infarction and for atrial fibrillation. In the vast majority of cases, these drugs will have established safety profiles and dosing regimes and would not be expected to induce adverse effects due to toxicity. However, in some cases, we may use novel compounds. In these instances, we will first establish appropriate dosing regimes and safety profiles in separate studies. There is a possibility these evaluation studies could reveal unexpected toxicity effects and if observed, this would mark the end of these experiments (humane killing and tissue harvest) and as such should not result in lasting harm or discomfort to the animals.

In some studies we will evaluate why some commonly used drugs including antidepressants, anticancer and antifungal medications can increase the risk of abnormal heart rhythms in patients. Here, if adverse effects did develop we would expect these to result in the rapid loss of consciousness and death. If this occurs, we would expect this to happen over a just a few seconds and, due to the rapid loss of consciousness, not result in lasting pain or discomfort.

In all animals where we obtain blood samples, measure the ECG and blood pressure we would only expect mild and transient discomfort lasting a few seconds associated with, typically, insertion of a needle or catheter to take blood samples or administer substances.

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

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

Mild - 35%

Moderate - 65%

What will happen to animals used in 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?

Diseases of the cardiovascular system such as heart failure, heart attacks and abnormal heart rhythms do not occur in isolation in the body. For example in heart failure where the heart fails to pump blood effectively, the reduction in blood flow to peripheral organs such as the kidney or carotid body result in the reflex release of hormones and altered nerve signals - the neurohumoral response. The neurohumoral response then also alters the function of the heart and other organs in the body which in turn also feedback to further modify heart function. This complex interplay between the heart, nerves, hormones, other organs and even the immune system during disease is not yet faithfully reproduced by any computer model or cellular models meaning that studies of this nature still need to be completed in living organisms. Studies using patients with cardiovascular disease are potentially of considerable value as the heart dysfunction exists in the context of interactions between organs. However, there are major limitations associated with using the 'human model' including not least of all the use of many different drugs to try and manage the disease, poor cohort consistency and the presence of other diseases or comorbidities.

Cellular models such as stem cell heart models, 2-dimensional and even 3-dimensional organoids (organs on a chip) have advanced considerably in recent years but do not replicate the important aspects of organ-cross talk in cardiovascular diseases. In addition, a large body of work including our own, has shown the importance of cellular architecture in determining heart function in health and disease. Critically cellular structure in stem cell heart models and thence 2d and 3d organoids is fundamentally different to that in the adult heart and as such their applicability in disease modelling and assessment of therapeutic interventions remains limited.

In silico or computer models are becoming more powerful and today can reproduce key aspects of the mechanics of the whole heart and have some predictive value regarding the impact changes to the electrical properties of the heart. Nevertheless, they still have significant constraints that limit their use when studying long term conditions such as heart failure. Amongst these is they do not sufficiently model cross talk between different cell types in the heart nor between the heart and other organs; they

tend to be simplistic in their approach to how signalling pathways modulate heart function and treat the 'cell' as a homogeneous entity when in reality there are dozens of enzymes and proteins controlling the amount, localisation in the cell and timing of responses; they are not able to integrate the complexity of inputs to and outputs from the heart in a whole body systematic approach over the timeframes that occur during the development of cardiovascular diseases.

Simpler model organisms such as zebrafish and fruit flies are useful tools for screening and early hypothesis testing but have several major limitations not least of which is their cardiovascular system is very different to that in human and the structure of their heart cells is fundamentally different lacking the specialised membrane structures pivotal to the function of the heart in mammals. Nevertheless, many of our experimental decisions are informed by experiments that we have (or will have) performed using non animal alternatives including cell lines, stem cell models and lower organisms including fruit flies before undertaking regulated procedures in protected species.

We have evaluated the available alternative models listed via NC3Rs and the EU Joint Research Centre Data Catalogue and these are typically computer model or hiPSC based and therefore, whilst informative, suffer from the limitations noted above.

Finally, a key element of the programme of work is to evaluate the response to novel therapeutic interventions in relevant models of human disease. This both needs an intact organism level approach to be successfully delivered and, additionally, this *in vivo* methodology is a key translational regulatory step.

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

- Immortal cell lines
- hiPSC cardiac myocytes
- 2d and 3d organoids
- Computer models

Why were they not suitable?

Immortal cell lines: With few exceptions, these are not cardiac in origin. Lines of cardiac origin lack the 3d ultrastructure of adult cardiac myocytes which we know is an important factor when considering mechanisms regulating cardiac function in health and disease

hiPSC cardiac myocytes: These also lack the ultrastructure of adult cardiac myocytes. Additionally, unlike adult cardiac myocytes they do not have a stable resting membrane potential.

2d and 3d organoids: Again have major structural differences with adult cardiac myocytes and poorly replicate the cell to cell connectivity seen in the adult heart between muscle cells and between muscle and adjacent non muscle cells. They also lack the intact innervation system seen in the adult heart

Computer models: Do not have the required level of fidelity to replicate the true structure of each cell in the heart nor the compartmentalisation of different signalling molecules in heart cells.

In addition to the specific limitations noted above, the diseases which we aim to study are multi-system diseases, these cannot be modelled *in vitro* in cells nor organoids and, computer models are still of limited use in this context. Moreover, we are studying the long term effect of novel therapies for cardiac diseases, none of the alternative models are capable of replicating the *in vivo* responses which are critical to understanding the efficacy and mechanisms of effect of these therapeutic approaches.

Reduction

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

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

The numbers of animals to be used are based on historical cohort sizes from our own previous studies (key publications in 2009, 2011, 2014 and 2019 as examples). These studies have shown consistent and reproducible biological responses with similar group sizes.

Historical data are used to characterise expected between- and within-animal variability for the key physiological, structural and functional endpoints that underpin the project. On this basis, animal numbers are set at levels that have previously been sufficient to support clear biological interpretation and experimental decision-making.

Animal numbers are therefore determined by pre-defined scientific decision thresholds, rather than formal statistical detectability. These thresholds relate to whether observed effects are consistent in direction and magnitude across animals, align with established disease mechanisms, deviate from normal physiological ranges and are sufficient to justify progression, refinement, or discontinuation of a given experimental approach. If expected disease-associated changes are not reproduced reliably in the model, further use of animals under that protocol step is discontinued. As a specific example in cardiac disease modelling, continuation of an intervention or progression to subsequent study phases requires consistent changes across animals in integrated cardiac function, such as ventricular contractility, relaxation, or electrical stability, that are clearly distinguishable from baseline variability observed in healthy or sham-treated sheep.

Where appropriate, outcomes are reviewed against these criteria during the course of the work to ensure that animals are not used unnecessarily. All estimates and design decisions are informed by analysis of historical data and are reviewed with input from an independent external biostatistician.

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

Animal numbers are reduced through design choice. Wherever possible, longitudinal within-animal designs are used so animals act as their own controls, reducing between animal variability effects and thus the required group sizes required to address experimental hypoptheses. Shared control groups

and, where applicable, historical control datasets (matched for e.g. strain, age, housing and operating procedures) are used to avoid duplication. Sample sizes are set *a priori* using pilot and legacy data to inform realistic effect sizes and account for variance effects between and within animals anda are reviewed with support from an independent biostatistician. Experiments are staged with predefined progression criteria (e.g. a decrease in contractility or ventricular dilatation in heart failure with reduced ejection fraction studies) to prevent unnecessary use of animal numbers in an uninformative study. The true experimental unit is defined at design stage to avoid pseudoreplication, and inclusion/exclusion criteria, randomisation and blinding are specified in advance to minimise bias and variance. We will use experimental design tools and statistical packages to check validitiy of group structure, allocation and analysis plans where appropriate.

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

The programme of work supports a number of principal investigators and their groups. It is standard practice in these groups that all animal resources are used as widely as possible. For example, we maximise tissue use and groups studying the different chambers in the heart and the carotid body will all share tissue from each animal. This prevents the need for separate groups to require their own cohorts of animals and markedly reduces our overall animal requirements as a result.

Our work is both informed and complemented by alternative methodologies. For example we have used Drosophila and cell lines as screening tools to identify candidate pathways *before* beginning *in vivo* studies to evaluate hypotheses.

In addition to extensive sharing of *in vivo* data and tissue sharing for *in vitro* experimentation, we also bank tissue and liquid samples at the time of tissue harvest for the use in subsequent biochemical and imaging experiments.

Breeding strategies are not relevant to the present programme of work.

Equipment is serviced and maintained to ensure measurement methods are as reproducible as possible between animals and experiments.

Standard operating procedures are followed to minimise inter and intra operator variability.

Standardised operating procedures, cohort demographics (e.g. age, strain), regular equipment servicing and training ensure measurements are consistent across animals and experiments, lowering unexplained variance.

Reduced variance arising from the use of standardised procedures increases statistical power for a given effect size, meaning fewer animals are required per study and reducing the risk that experiments must be repeated due to ambiguous or underpowered results.

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 programme of work will use several different models of heart disease that closely resemble the commonest cardiac diseases in man, including heart failure, myocardial infarction and atrial fibrillation. All of the surgical procedures that we will use are refined to be as minimally invasive as possible and require only a small incision in the skin over the neck to gain access to blood vessels through which all procedures are then performed. The surgical devices and techniques that we use are identical to those used clinically in patients where they are associated with rapid post-operative recovery and minimal patient discomfort.

Following recovery from surgery, animals will be monitored closely for the development of clinical signs of heart failure that can include breathlessness at rest, coughing and reduced activity. These measurable signs of heart failure as humane endpoints are necessary as a key aim in our studies is to meaningfully evaluate new treatment approaches for heart failure. If we do not allow these signs to develop, it would be impossible to know firstly if we have developed clinically relevant models and therefore secondly, whether these new therapies would be effective in the clinical population.

Animals will also undergo additional investigative procedures including echocardiography, blood pressure and ECG measurements. These will be performed in conscious animals using the same methods used in human and only require gentle manual restraint to be completed successfully. These measurements are not associated with any lasting harm or discomfort.

For some investigations, animals will be placed under general anaesthesia and we will place a catheter into a blood vessel accessed by placing a needle through the skin directly into the blood vessel. We will then use this catheter to introduce leads for measuring the electrical activity and pressures inside the heart. Again, this methodology and equipment is used clinically in patients undergoing the same type of investigation and is associated with minimal post operative discomfort or harm. These direct measurements from within the heart provide much more detailed information on the electrical performance of the heart than can be obtained using just skin electrodes and, as such, provide better understanding of the mechanisms that are altered in disease and help identify and evaluate new treatment approaches with much greater accuracy.

Given the minimally invasive surgical and non-surgical approaches and non-invasive assessments in conscious animals we expect pain and discomfort due to the procedures to be minimal and transient in nature. However, given the requirement for animals to recover from anaesthesia and progress toward the development of heart failure, with careful monitoring the overall suffering is expected to not exceed a moderate level. Due to the objectives and hypotheses the programme of work is addressing, there is a requirement for animals to progress toward the development of heart failure. Nevertheless, the combination of minimally and non-invasive approaches and careful monitoring minimises the suffering and distress as much as is practicably achievable.

Earlier humane endpoints (i.e. termination prior to the development of measurable clinical signs of heart failure) are not viable because the primary aim of the programme is to study mechanisms and treatments relevant to established human disease. Structural, electrical and autonomic abnormalities that define e.g. heart failure—and determine therapeutic efficacy—only emerge once a defined level of functional impairment is present. Ending procedures earlier would prevent confirmation that a clinically relevant disease state had been achieved and would make it impossible to determine whether proposed interventions modify disease progression rather than only early or subclinical changes. This would risk generating data that are not translatable to patients and would likely necessitate additional repeat studies, increasing overall animal use.

All operators are closely supervised and trained in all procedures and will not undertake unsupervised activities until deemed fully competent.

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

Heart failure, atrial fibrillation, many arrhythmia syndromes and myocardial infarction are diseases that occur in adults with much higher prevalence than in children. As such, to achieve the most translationally relevant models, we conduct experiments in fully grown animals. In addition, undertaking experiments in adult animals removes two significant limitations to the applicability of our programme of work to the clinical conditions we are investigating. Firstly, the structure of cells in the adult heart is profoundly different to that in the immature heart and secondly, the rapid growth phases in immature animals significantly confound interpretation of data between groups and complicate implantation of devices such as pacemakers where the leads placed into the heart would need continual replacement as animal size increases.

Rodents, Zebrafish and fruit flies are useful model organisms. We will continue to use fruit flies and cells derived from rodents not having undergone any regulated procedures to test hypotheses and evaluate drugs before conducting experiments *in vivo* using regulated procedures in sheep. However, there are considerable limitations across these species including profound differences in cardiac anatomy, cellular structure, electrical properties and response to various stimulants used to evaluate cardiac physiology. Whilst these model organisms can be used to inform experiments and in some cases be used as higher throughput screening tools, these differences mean that they are inappropriate choices to fully address our objectives and specific hypotheses.

The development of heart failure or remodelling of the heart following myocardial infarction or during atrial fibrillation usually occurs over a period of several weeks and as such it is not possible to complete experiments entirely in terminally anaesthetised animals. Nevertheless, where we require final assessments of cardiac function requiring anaesthesia, we do complete these under terminal anaesthesia prior to humane killing and harvesting tissues for laboratory based experiments.

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

All animals are monitored by both the research team members and animal care technicians / NACWO. Personnel are trained in the signs associated with pain, discomfort and development of heart failure. When these signs are first noticed this triggers interventions including consultation with named staff, enhanced frequency of monitoring and appropriate use of pain controlling drugs.

We have, and will continue to do so, refined our surgical approaches and are now exclusively using minimally and non-invasive approaches to complete surgical steps and monitor cardiac function. These steps reduce the likelihood of post-operative incidents that may require extended pain control measures.

Animals are habituated to handling and are housed in social groups apart from the immediate post operative period when they are singly housed in sight and sound of their home cage. As soon as the animals have recovered from general anaesthesia and they are deemed fit, they are returned to their social housing. These approaches reduce environmental stresses associated with handling, surgery and non-invasive monitoring procedures as much as is practicably possible.

Animals are provided with environmental enrichments including rubbing posts, toys such as plastic balls and puzzle feeders

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

We will monitor the published literature in scientific and learned society journals (e.g. LASA) and online resources from sites including NC3Rs, FELASA, LAVA, RCVS and EU Joint Research Centre to remain up-to-date with respect to best practices and developed refinements and alternative methodologies.

We follow the concepts behind the PREPARE and ARRIVE2 guidelines when planning experiments submitting findings for publication.

Experimental plans are submitted for Named Persons approval before commencing.

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

Remaining informed of and adopting relevant advances is core to how we operate. The animal care facility holds several 3Rs lunch and learn workshops each year, our research divisions hold regular seminars and we routinely attend scientific conferences where recent advances in methodology are presented and discussed.

We also participate in welfare related events and conferences on alternative methodologies including recently presenting at an RSPCA focus on severity workshop and showcasing the use of Drosophila as a screening tool for cardiovascular research at international conferences.

Through weekly laboratory meetings we update group members on recent publications and communications at conferences.

The investigators also serve on a number of committees, grant panels and editorial boards where animal welfare and experimental approaches are shared to promote wider adoption where appropriate

We will also routinely conduct web and database searches and screen recent publications and Learned Society newsletters to ensure we remain abreast of developments in 3Rs space and implement changes as appropriate in our studies.