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Project Titles and key words

- Rodent regulatory genotoxicity

  Genotoxicity, Cytoxicity, Rodents

- Non-clinical studies in domestic livestock species

  Safety, Efficacy, Surgical models, Livestock species

- The role of inflammation in efficacy and safety

  Inflammation, Efficacy, Pharmacology, Safety

- Rabbit irritancy and toxicology studies

  Rabbit, Toxicology, Safety assessment

- Cardiovascular, respiratory and related pharmacology

  Cardiovascular, Respiratory, Safety, Efficacy

- Resolution of inflammation & macrophage biology

- Local cyclic nucleotides dynamics and their role in heart disease

  cAMP, heart failure, cardiac hypertrophy

- Deciphering Neural Crest Gene Regulatory Network

  Neural crest, zebrafish, gene regulatory circuitry

- Development of an allogeneic aortic graft

  Tissue Engineering, vascular bypass graft

- Evaluation of Toxic Hazards to Fish

  Ecotoxicology, freshwater, marine, fish

- Neuronal function and psychiatric disorders

  Autism, schizophrenia, neuronal activity, plasticity

- Effects of phytase in farm production

  Efficiency, environment, enzyme, pig, nutrients

Rodent regulatory genotoxicity
Genotoxicity, Cytoxicity, Rodents

- Summarise your project (1-2 sentences)

The project aim is the determination of scientific and/or regulatory endpoints in rodent genetic toxicology tests for submission to regulatory authorities and/or for safety assessment purposes.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

Governments require and the public expects that substances (e.g. agrochemicals, industrial chemicals, pharmaceuticals, medical devices, microbes used as pest control agents (MPCAs) and oncolytic viruses used to kill cancer cells) that we are potentially, or actually, exposed to are safe or their hazards are well understood.

- Outline the general project plan.

The preliminary toxicity tests, micronucleus test, comet test and UDS assay in this project are designed determine specific genotoxicity, cytotoxicity or regulatory endpoints and/or for safety evaluation.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

The procedures performed include the administration of substances by various routes (e.g. oral or injection). In addition to the findings indicated above, occasionally effects may occur which are expected due to the nature of the test material (e.g. pharmaceuticals), but they are not expected to persist for longer than a 24-hour period. Most of the dosing techniques, manipulations or investigations do not cause any lasting adverse effects, but a small number of animals may show temporary moderate distress due, for example, to restraint, confinement and withdrawal of blood.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

The information gained from the studies performed under this project can be used by medical, health and safety practitioners, and toxicologists etc. to assess the relative safety of the substances being used, abused or handled and therefore develop appropriate strategies for the treatment or safe handling of the substances.

In addition, the information can be used to assist in the selection of dose levels for repeat dose studies in rodents and non-rodents with a higher degree of confidence and therefore minimise animal use and the severity of findings in later studies. Study designs can therefore be developed that cause the least pain, suffering, distress or lasting harm and which have the highest prospect of achieving the desired scientific endpoints.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

There is currently no regulatory or scientifically acceptable alternative to the use of animals in these studies. Rodents are used as they are required and accepted by the regulatory authorities for these study types. Approximately 3500 rats and 2500 mice will be used of the 5 year duration of this project license. The regulatory guidance usually indicates the number of animals included in a study; otherwise, the number used is the minimum to achieve the aims of the study.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

There is currently no regulatory or scientifically acceptable alternative to the use of animals in these studies. Rodents are used as they are required and accepted by the regulatory authorities for these study types.

- Explain why the protocols and the way they are carried out should involve the least suffering.

To prevent unnecessary pain and suffering to animals and refine the studies, a tiered approach to safety testing is generally adopted. All available information will be reviewed to decide whether testing is acceptable. If acceptable, a logical sequence to testing will be determined. The majority of animals on these studies would be expected to experience no effects or those of a mild to moderate severity during the dosing and/or observation phases of the study. However, in order to achieve scientific and regulatory objectives in the preliminary toxicity tests, some animals may show severe effects (such as overt clinical signs, effects on bodyweight) and/or mortality.

Safety, Efficacy, Surgical models, Livestock species

- Summarise your project (1-2 sentences)

This project enables a range of efficacy, regulatory safety/toxicity and supporting studies in domestic livestock species.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

Governments require, and the public expects, that substances/articles to which humans or domestic animals may be exposed are effective and safe and/or well-characterised. Therefore, new substances must be evaluated before they are made widely available for use; this is a mandatory legal requirement which requires the use of animals in studies to evaluate systemic exposure, efficacy and toxicity.

- Outline the general project plan.

Many studies conducted under this project are designed to assess efficacy and/or safety of veterinary medicines and other products in the species that will be exposed to such products through their intended use, i.e. the target species. It is a regulatory requirement that safety data in the target species must be generated to support further development, veterinary clinical trials and ultimately marketing approval of the product. Likewise, regulatory requirements exist for data generated in food producing livestock species which are indirect 'target species' at risk of exposure to agrochemicals/other potential environmental pollutants.

This project also includes studies in which livestock species are used as models for the assessment of human safety, including toxicity and surgical models.

Testing programmes are performed sequentially, with review of findings at each stage.

Studies vary in duration from a single dose to daily dosing for up to 12 months, depending on the intended/likely duration of human or target animal use/exposure. The numbers of animals used are kept to the minimum commensurate with meeting study objectives.

On study completion, some animals may be re-used in another study, but most animals are humanely killed by anaesthetic overdose to allow examination of the organs/tissues. However, where terminal investigations are not required and no other regulated use is planned, dogs, cats and horses are re-homed as companion animals whenever possible, following careful assessment for suitability/compatibility of both the animal and the carer.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

Animals are dosed/treated by the intended/likely route of human (or target animal) exposure, and observed regularly to monitor appearance, behaviour and clinical health. Typical investigative procedures are similar to diagnostic procedures that might be used medically to monitor progress of a human patient (e.g. collection of blood samples for laboratory investigations, or ECG monitoring to assess heart rate/function).

Most animals are expected to experience no adverse effects, or only mild effects such as slight weight loss. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss or reduced activity, and a very small number of animals may potentially experience severe adverse effects without intervention. However, humane end-points are applied to prevent unnecessary suffering.

Animals in surgical studies may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients; for example, in the case of renal studies, animals may experience some degree of impaired renal function which could potentially lead to kidney failure without appropriate interventions. However, supportive treatments are given to eliminate or minimise these adverse effects, and humane endpoints are again applied. All surgical procedures are performed under anaesthesia, with full peri- and post-operative analgesic cover.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

The principal benefit of the project is the provision of data to facilitate sound regulatory decisions on e.g. clinical trial approval or marketing authorisation for new medicines or other substances or articles to which humans or domestic animals will be exposed, thus contributing to their protection and safety.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

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

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

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

The numbers of animals used are kept to the minimum commensurate with meeting study objectives, through careful assessment of results at each stage of testing, reference to all available sources of information on the test article under evaluation, compliance with guideline recommendations on minimum group sizes where applicable, and the appropriate use of statistical principles in study design.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

Although non-animal (*in vitro*, *in silico*) studies can provide useful supporting data to refine and reduce animal studies, definitive assessments of systemic exposure, efficacy and toxicity can only be achieved in studies using intact animals, and this remains a mandatory legal requirement; currently, there are no scientifically, ethically or legally acceptable non-animal alternatives available.

- Explain why the protocols and the way they are carried out should involve the least suffering.

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

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

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

The role of inflammation in efficacy and safety
Inflammation, Efficacy, Pharmacology, Safety .

- Summarise your project (1-2 sentences)

The aim of this programme of work is to determine the efficacy and safety of new treatments for human inflammatory diseases, including respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD), and rheumatological disease, including arthritis.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

As of 2011, approximately 235 million people worldwide were affected by asthma and approximately 250,000 people die a year from the disease. Mild to moderate asthma is well controlled with inhaled bronchodilator/corticosteroid combinations. Severe asthma, however, is poorly controlled with this treatment and maintenance and prevention of exacerbations remains an unmet clinical need. COPD is projected to become the fourth leading cause of death worldwide by 2030 and is already the third leading cause of death in the U.S. COPD is poorly controlled by current therapies and new drugs are required that prevent the progression of air flow limitation, induced by the inhalation of noxious gases. Arthritis is a common condition that causes pain and inflammation within a joint. Approximately 10 million people in the UK suffer from the disease. The condition affects people of all ages including children. Types of arthritis affect different tissues in the body including osteoarthritis, lupus, gout and rheumatoid arthritis, all resulting in the reduction in movement and the breakdown of bone and cartilage. There is no cure for arthritis but there are a number of treatments that can help slow down the condition's progress. Governments require and the public expects that medicines are safe and/or well-characterised. Therefore, before humans are exposed to new substances, their safety must be evaluated; this is a mandatory legal requirement. This safety assessment requires the use of animals in studies to evaluate systemic exposure/toxicity; currently, there are no scientifically, ethically or legally acceptable alternatives available that do not involve the use of animals.

- Outline the general project plan.

This project licence will permit the development and subsequent testing of potential new medicines in animal models that mimic some aspects of inflammatory disease. The compounds potential to prevent or reverse measures of inflammation will be measured. In some circumstances potential side effects will also be measured in the same animal models. Results from these models will help predict how effective they will be in clinical trials and help prevent compounds with unwanted effects reaching the clinic. Potential new medicines will have undergone extensive testing in cell cultures before being selected for testing in animals. Mechanisms driving efficacy and safety are complex and currently data from animal models are still required for regulatory authorities before potential new medicines reach the clinic.

A mild inflammatory response will be pharmacologically or physically induced in a similar way to humans either before, after or during a potential medicine is given. The induction of the inflammatory response may be on a single occasion or repeated over a period of time. Measures of the inflammation will be assessed and the effect of the compound compared to that of the animals not receiving compound. Comparative materials may be

used, such as marketed products in order to demonstrate a likely advantage of the novel test substance over current materials in the clinic.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

The induction, assessment and resulting inflammation will cause no or mild adverse effects such as slight weight loss. A small percentage of animals may show more significant adverse effects indicating moderate severity, e.g. more marked weight loss or reduced activity. To limit the animals discomfort, additional bedding, provision of moistened food within the floor of the cage, longer sipper tubes on water bottles will be provided. Specialist veterinary staffs are always available to advise and assist in the welfare of the animal. Humane end-points are applied, under veterinary guidance as necessary

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

Novel compounds that reduce inflammation with minimal side effects in these studies may be progressed into longer term toxicology studies before being presented to the regulatory bodies for approval for use in the clinic.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

Mice and rats will predominantly be used in these studies. These species are used because they respond to inflammation in a similar manner to the humans and the data produced will help predict how well the potential medicines will work in humans. In a limited number of studies, guinea pigs, rabbits, ferrets or dogs may be used.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

The number of animals used will be kept to a minimum and statistical analysis will be used to determine the number of animals required per group to allow a meaningful outcome. Experimental designs are constantly reviewed and alternative cell assays considered as technology improves, however due to the complex nature of the inflammation pathways there are no current alternatives to use animals.

- Explain why the protocols and the way they are carried out should involve the least suffering.

Wherever possible, experimental end points are collected under anaesthesia or post mortem to reduce the burden on the animal used in the protocols. In some circumstances safety markers will also be collected from the animal models maximizing the data from individual studies.

Rabbit irritancy and toxicology studies
Rabbit, Toxicology, Safety assessment

- Summarise your project (1-2 sentences)

This project's objective is provision of non-rodent data for regulatory submission and/or for safety assessment purposes with the rabbit being the regulatory accepted test species. The data will be used to review substances under development and, where appropriate, satisfy governmental regulatory requirements necessary to gain clinical trial approval and/or marketing authorisation.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

Governments require and the public expects that substances we are exposed to are safe or their hazards are well understood. Regulatory approval is required to allow drugs to be tested in human or veterinary trials, or for chemicals, agrochemicals, food additives/substances or medical devices/articles to be marketed.

- Outline the general project plan.

Studies conducted in this project form part of a framework of studies designed to investigate potential effects of pharmaceuticals, chemicals, agrochemicals, food additives/substances or medical devices/articles to facilitate a review of substances under development and, where appropriate, satisfy governmental regulatory requirements necessary to gain clinical trial approval and/or marketing authorisation.

Regulatory testing requirements generally follow a tiered approach, with the extent dependent on the intended use of the chemical and its stage of development. Studies are designed to determine specific toxicity or regulatory endpoints, and/or for safety assessment, ranging from single-dose toxicity, irritancy or local tolerance studies to repeat-dose studies.

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

Studies are performed stepwise to have the highest prospect of refining and optimising the programme to achieve the necessary scientific endpoints whilst resulting in the least pain, suffering, distress or lasting harm to the animals. Preliminary studies are performed on the basis of limited information (from various sources including previously tested structurally similar chemicals and in vitro and other studies) and there may be uncertainty regarding the severity of the response. In subsequent studies, the majority of animals would experience no effect or those of mild or moderate severity. A range of dosages is given to different groups of animals to determine a dose-response relationship for any adverse effects seen. For scientific and regulatory reasons it is important that a "no observable adverse effect level" is determined, therefore, the highest dose may cause effects.

Animals are treated by the intended/likely route of human (or target animal) exposure, and observed regularly to monitor appearance, behaviour and clinical health. Most animals are expected to experience no adverse effect, or only mild effects. A small percentage of animals may show more significant adverse effects indicating moderate severity and a very small number of animals may potentially experience severe adverse effects without intervention. Humane end-points are applied to prevent unnecessary suffering.

Animals in surgical studies may, as a result of the surgical procedure, experience some adverse effects similar to those that might be experienced by human patients, however, supportive treatments are given to eliminate or minimise these adverse effects, and humane endpoints are again applied. All surgical procedures are performed under anaesthesia, with full peri- and post-operative analgesic cover.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

The principal benefit of the project is the provision of data to facilitate sound regulatory decisions on e.g. clinical trial approval or marketing authorisation for new medicines or other substances or articles to which humans or domestic animals will be exposed, thus contributing to their protection and safety.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

The rabbit shares many characteristics with rodents and is classed as a non-rodent; it is acceptable to the regulatory agencies in situations where a second and/or alternative mammalian species is required and is the regulatory species of choice for irritation and local tolerance studies.

Regulatory testing requirements generally follow a tiered approach, with the extent dependent on the intended use of the chemical and its stage of development. Studies are designed to determine specific toxicity or regulatory endpoints, and/or for safety assessment, ranging from single-dose toxicity, irritancy or local tolerance studies to repeat-dose studies.

The numbers of animals used are kept to the minimum commensurate with meeting study objectives, through careful assessment of results at each stage of testing, reference to all

available sources of information on the test article under evaluation, compliance with regulatory guideline requirements on group sizes and, where applicable, the appropriate use of statistical principles in study design.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

Although non-animal (*in vitro, in silico*) studies can provide useful supporting data to refine and reduce animal studies, definitive assessments of systemic exposure, efficacy and toxicity can only be achieved in studies using intact animals, and this remains a mandatory legal requirement; currently, for many of the study types in this project, there is no scientifically, ethically or legally acceptable non-animal alternative available.

For irritation studies a weight-of-evidence analysis including assessment of physiochemical characteristics of the test substance, data from *in vitro* alternatives and, where available, *in silico* investigations. When regulatory acceptable, irritation studies will be conducted by an appropriate *in vitro* method.

- Explain why the protocols and the way they are carried out should involve the least suffering.

Studies are performed stepwise, with a review of findings at each stage and modification of subsequent stages as necessary, to have the highest prospect of refining and optimising the programme to achieve the necessary scientific endpoints whilst resulting in the least pain, suffering, distress or lasting harm to the animals. Animals are monitored for clinical signs of toxicity or other effects on their health and wellbeing, and in order to prevent unnecessary suffering, humane end-points are applied.

Preliminary studies are performed on the basis of limited information (from various sources including previously tested structurally similar chemicals, in vitro and other studies) and there may be uncertainty regarding the severity of the response. In subsequent studies, the majority of animals would experience no effect or those of mild or moderate severity. A range of dosages is given to different groups of animals to determine a dose-response relationship for any adverse effects seen. For scientific and regulatory reasons it is important that a “no observable adverse effect level” is determined, therefore, the highest dose may cause effects. Local tolerance studies are conducted on clinical preparations to assess effects at the site of administration and an evaluation is made of any potential local reaction in the event of clinical mis-administration. Studies are also conducted that mimic the effects of accidental human exposure of chemicals.

Cardiovascular, respiratory and related pharmacology
Cardiovascular, Respiratory, Safety, Efficacy.

- Summarise your project (1-2 sentences)

This licence is being used to assess the safety and efficacy of test materials in cardiovascular, respiratory and other related systems in rodent and non rodent species.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

Governmental approval is required before a new chemical may enter clinical human or veterinary trials, food products, or the workplace and further approval may be needed before it can be marketed. Guidelines issued by government appointed regulatory agencies specify the types of animal and non-animal studies that must be completed in order to apply for clinical trial authorisation or product marketing approval. In addition to the safety requirements detailed above, for Pharmaceuticals, there is also an expectation of efficacy i.e. that the novel drug actually produces the beneficial effect for which it is entering the clinical trial or marketplace.

- Outline the general project plan.

Safety Testing programmes include a mandatory examination of test materials on the Cardiovascular, respiratory and CNS and other systems if a risk to their function is identified, prior to evaluation in man. This programme of work is associated with examination of test materials on the heart and blood vessels, lungs, kidneys and the blood to achieve the desired scientific endpoints with the least risk of pain, suffering, distress or lasting harm to the animals. Scientific endpoints are quite rigid and defined in international guidelines for each particular organ system (e.g. Effects on the ECG, blood pressure and heart rate for the cardiovascular system, and a measurement of respiratory rate, tidal volume and minute volume to evaluate any effects on the lungs).

Efficacy programmes have less rigid endpoints, and may be dependent on the particular target of a test material, but will generally be similar to those needed in safety testing.

Studies vary in duration from a single dose to daily dosing for up to 28 days, depending on the intended/likely duration of human use/exposure. Numbers of animals used in each study are generally linked directly to those in the published regulatory guidelines. Animal numbers are kept to the minimum commensurate with meeting study objectives.

Animals are dosed by the intended/likely route of human exposure, and observed regularly to monitor appearance, behaviour and clinical health. Investigative procedures carried out in these studies are similar to diagnostic procedures that might be used medically to monitor progress of a human patient and include, for example, collection of blood samples for laboratory investigations, or ECG monitoring to assess heart rate/function.

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

- Predicted harms: Give a brief description of the procedures to be applied to the

animals used in this project and describe the expected adverse effects.

Animals may be implanted with devices to allow continuous recording of cardiovascular parameters. These surgeries will usually be performed by trained people (including veterinary surgeons) under general anaesthesia utilising aseptic techniques, with appropriate antibiotics and pain relief, and an appropriate recovery time. Such animals may be used on several studies, with appropriate time in between, only with the approval of a veterinary surgeon. Animals on these study types will be dosed with test materials and carefully monitored for adverse effects during testing.

Animals on this programme of work that are not implanted surgically will generally undergo acute rather than chronic testing, mainly involving a single dose of test material, and will be similarly monitored closely for potential adverse effects.

Any animals showing adverse effects to test materials will be monitored more closely than normal. Veterinary advice will be taken regarding any treatment needed to stop any adverse effects occurring, including the cessation of dosing.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

Identification and characterisation of effects on Cardiovascular, Respiratory and Related function and accurate profiling of side effects on these systems in terms of nature, severity and duration of effect will contribute directly to the drug discovery and development process by enabling drugs with the least adverse effect on these organ systems to be taken forward for further development, in further animal testing and human clinical trials. Data generated from these studies for compounds which proceed to clinical development may form part of the regulatory submission. By conducting such testing, ultimately, marketed pharmaceuticals may become available which improve human health and do not have adverse side effects. Another important output of this type of testing is to identify compounds that are not suitable for further pre-clinical and clinical development due to unacceptable side effect profiles which will reduce the total number of animals tested as further development of the test substance will be terminated.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

It is impossible to be precise regarding the number of animals that will be used on this project, but we will use the following principals.

All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals. For study types that are less well established and for which historical data may not be available, the literature will normally be consulted to help establish the group size. Statisticians are often consulted particularly where the study type is not routine.

Where possible, common control groups will be used wherever possible in order to minimise the numbers of groups used.

Rodents and guinea-pigs will be mainly used for the tests conducted under this licence. These species are considered to be of the lowest neurophysiological sensitivity commensurate with achieving the study aims. However, in some safety and efficacy studies, other species may be used due to their similarity to man for the target being

tested (e.g. an ion channel which is responsible for a fatal heart disturbance in humans) or because of regulatory requirements for a non rodent species for testing a new test material.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

The intact Cardiovascular, Respiratory and Renal systems are complex systems which are not fully understood and therefore for the protocols listed in this project, there are no adequate models to replace the whole animal experimental model, as the complex mechanisms under investigation cannot be adequately modelled in non-sentient preparations.

- Explain why the protocols and the way they are carried out should involve the least suffering.

Protocols are carried out in a stepwise fashion only using techniques and procedures necessary to achieve the objectives. Humane endpoints are detailed in the programme and if they are in danger of being exceeded, remedial action will be taken to prevent any further suffering for any animals.

Resolution of inflammation & macrophage biology

- Summarise your project (1-2 sentences)

Chronic inflammation drives the development of diseases such as rheumatoid arthritis, which affects 1-2% of the UK population and cardiovascular disease - the leading cause of premature mortality and morbidity in an increasingly elderly population. The aim of this project is to develop a better understanding of the inflammatory response and macrophage biology to identify endogenous pathways and mediators that represent valid targets for the development of new classes of anti-inflammatory drugs.

- Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.

This work is needed because the cells and molecules that regulate the resolution of inflammation are less well understood than the processes that initiate inflammatory responses. All classes of anti-inflammatory drugs currently used in the clinic have significant limitations or unacceptable side-effect profiles in long-term treatment protocols. Identification of novel targets for therapeutic intervention in chronic inflammation will improve treatments for a significant number of patients whose lives are diminished by chronic illness.

- Outline the general project plan.

This project will use genetically modified animals to follow the recruitment of white blood cells called monocytes into sites of inflammation and their differentiation into specialised cells called macrophages that contribute significantly to the development of chronic inflammation and the initiation of tissue repair. Using well characterised transgenic and knockout strains of mice will allow us to examine the role of specific mediators and receptors in the process of inflammation resolution. Testing chemicals and peptides that activate or inhibit specific macrophage receptors will allow us to validate specific pathways in the process of inflammation resolution as targets for the development of novel anti-inflammatory drugs. To identify the role of key cell types in the regulation of inflammation we will use radiation chimeras and to identify the role of specific plasma proteins we will use hydrodynamic delivery protocols to deliver plasmid DNA or recombinant viruses to liver cells in vivo

- Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.

Briefly, the experimental protocols used in this programme of work involve initiating an inflammatory disease process locally or systemically by delivery of a well characterised non-infectious chemical stimulus and following the kinetics of inflammatory cell recruitment or testing the effect of novel molecules to modulate the inflammatory disease process in vivo. These experiments have a direct link to the development of better drugs for the treatment of chronic inflammatory diseases. We expect adverse effects to be minimal based on our extensive experience and our continuing commitment to animal welfare and application of the 3Rs.

To achieve a more detailed understanding of the key cell types involved in the regulation of the mammalian inflammatory response we will need to use genetic technologies to direct transgene expression to specific cell types using e.g. the use of mice with cell type specific expression of the Cre recombinase protein and for some experiments the use of radiation chimeras to allow transgene expression only in reconstituted cells of haematopoietic origin e.g lymphocytes and monocytes rather than stromal cells e.g. fibroblasts and endothelial cells. To study the role of specific proteins in the resolution of inflammation we will use hydrodynamic delivery of plasmid DNA or recombinant viruses to liver cells to obtain long-lasting levels of transgenic protein production.

Protocol 1 (mild) will be used to create new strains of genetic reporter mice by pro-nuclear injection of DNA into fertilised mouse eggs. Previous work from our group has used this technique successfully to derive lines of genetically altered mice that allow tracking of monocytes and macrophages at sites of inflammation. Protocol 2 (mild) will be used to induce superovulation in female mice to maximise the production of fertilised eggs for making transgenic mice. Protocol 3 (moderate) will be used to introduce injected eggs or blastocysts into pseudopregnant female mice to generate new strains of genetically altered animals. This protocol will also be used to recover strains of mice stored as frozen embryos. Protocol 4 (moderate) will be used to produce a small colony of vasectomised male mice that are necessary for generating and re-deriving strains of genetically altered animals. Protocols 5 (mild) and Protocol 9 (moderate) will be used to breed and maintain adequate stocks of genetically altered mice for use in models of inflammation.

Protocol 6 (moderate) will be used to study the recruitment and differentiation of inflammatory of cell types within the peritoneal cavity following injection of a standard inflammatory stimulus. This technique is widely used to study both acute and chronic inflammation and inflammation resolution and is particularly good for studying macrophage differentiation in vivo. In over 20 years experience of this protocol we have never seen animals suffer more than momentary discomfort following injection of substances. Protocol 7 (moderate) will allow the monitoring of inflammatory cell recruitment and inflammatory mediator production in a second anatomical site, namely a dorsal air pouch, this model is particularly good for studying early events in the elaboration of an inflammatory response such as neutrophil recruitment and activation. Animals will be briefly anaesthetised (less than 1 minute) to allow administration of air and substances subcutaneously. Protocol 8 (moderate) will allow us to follow systemic rather than localised inflammation following intraperitoneal or intravenous delivery of inflammatory mediators such as bacterial endotoxin or bacterial DNA or CpG. An important early read out of widespread inflammation is liver injury and changes in cytokine and biomarker expression in plasma. With our standard dosing regime animals do not suffer pain or distress.

- Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

The experiments in this project will advance the basic science of inflammation biology by identifying key pathways involved in the fundamental process of inflammation resolution and by giving us a better understanding of the molecules and pathways that regulate monocyte recruitment and macrophage differentiation. Work on the previous version of this licence identified two novel pathways of inflammation resolution. Novel drugs and peptides targeting these pathways of inflammation resolution have been identified and covered by UK and worldwide patent applications to facilitate further research work to be undertaken in collaboration with biotech and pharma companies. Publication of our research work and presentation of our findings at national and international meetings will improve the understanding of the basic biology of inflammation within the wider biomedical community and in the longer term we believe that this will improve treatment for thousands of patients affected by chronic inflammatory diseases.

- Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.

In addition to standard inbred strains of mice we will use lines of genetically altered mice that express easily detected reporter genes in specific cell populations. These unique

strains of mice will allow us to critically test the role of specific cell populations in simple models of inflammation initiation and resolution. To generate sufficient numbers of transgenic mice for our studies we predict that we will use no more than 3,500 mice per year on Protocols 5&9. Greater than 90% of the genetically altered animals used on this licence will be maintained on a mild severity protocol. Genetically altered mice and inbred strains of mice will be used in three different models of inflammation to better understand the endogenous pathways that regulate the magnitude and resolution of inflammation in either the peritoneum (Protocol 6, ~2,000 mice per year), a dorsal skin inflammation model (Protocol 7, ~400 mice per year) or systemic administration of inflammatory substances (Protocol 8, ~ 400 mice per year).

We constantly review our breeding programmes and experimental design to ensure that we obtain maximum information from the minimum number of animals.

- Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how you will use non-animal studies in parallel with the project.

Inflammation is a pathophysiological process that involves integration of multiple endocrine and immunological signals generated by multiple cell types can only be studied properly in experimental animals. Some aspects of inflammation biology can be studied ex vivo (e.g. cytokine production, cell adhesion under flow or chemotaxis) but in order to understand how these disparate aspects of cell biology are integrated in the context of a protective inflammatory response requires the use of a range of in vivo models of cell recruitment and inflammation.

Mice are the lowest vertebrate species that have the necessary range of inbred and genetically altered animals to achieve the overall aim of this programme of research. While some features of response to injury can be studied in flies and fish, these models do not have the monocyte subsets seen in human and murine blood and they do not have specialised populations of macrophages, neutrophils, T-cells, mast cells and dendritic cells, which we recognise as playing central roles in the mammalian response to infection and injury.

We will use cell lines and primary monocytes and macrophages obtained from blood and bone marrow to test the effects of new inflammatory mediators and immunomodulatory treatments before proceeding to in vivo studies. We constantly review the published literature on the development of alternatives to animal models and information relating to the application of the 3Rs including computer modelling.

- Explain why the protocols and the way they are carried out should involve the least suffering.

We constantly review our in vivo experimental protocols to ensure that they involve a minimum of suffering and we constantly review our analysis methods to ensure that we obtain the maximum amount of information and best readouts from every animal used in inflammation studies. Sharing of local expertise and best practice in surgical procedures has led to significant refinements in peri- and post- operative care and we constantly review the 3Rs literature.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Local cyclic nucleotides dynamics and their role in heart disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>cAMP, heart failure, cardiac hypertrophy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>five</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Over the last decade, landmark advances in the management of myocardial infarction (heart attack) have led to a significantly improved patients' survival but also to a proportionally larger population with a chronically diseased heart as a result of the initial lesion. It is well established that following a heart attack or in the presence of chronic high blood pressure the heart undergoes a progressive change in its structure and function resulting in increased size (hypertrophy), changes in the composition and organisation of cardiac tissue (cardiac remodelling) and progressively altered mechanical and electrical activity, eventually leading to heart failure (HF) and chronic heart rhythm disturbances, such as atrial fibrillation (AF). These conditions are estimated to consume ca. 4% of the annual NHS budget in the UK. These data underscore the major public health burden posed by HF and AF in our society and the need for a better understanding of the mechanisms that control such changes in cardiac structure and function.
A key molecule involved in cardiac remodelling is cAMP. cAMP is responsible for the appropriate reaction of the heart to hormones such as adrenaline, however it is also known to mediate adverse myocardial remodelling, through mechanisms that remain largely to be defined. A better understanding of how cAMP is involved in cardiac remodelling is a pre-requisite for devising more effective therapeutic strategies with reduced side effects.</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The primary purpose of the project is to advance biomedical science by increasing our knowledge of the role of the cAMP and of the enzymes controlling its intracellular levels (phosphodiesterases) in normal and diseased hearts. With this project we will be able to define a map of how cAMP changes in space and in time in cardiac myocytes in normal and disease conditions. Such a map is critical for our understanding of the regulation of cAMP signalling in defined subcellular compartments, to unravel how such signalling is altered in pathological conditions and to develop targeted therapeutic approaches.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a period of five years we expect to use approximately 11.000 mice and 2.500 rats.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The experiments we are proposing include characterising new genetically modified mouse models as well as generating mouse models of human disease, notably cardiac hypertrophy secondary to increased blood pressure on which we could also test new therapeutic strategies. Characterisation would typically include non-invasive (*e.g.*, echocardiography) and/or invasive (*e.g.* LV haemodynamics as terminal procedures) evaluation of cardiac mass and function under anaesthesia and ex-vivo investigation using myocardial tissue and cells. Mice might undergo voluntary exercise (by using a wheel available in their cage) or be administered agents that are used or considered for use in human disease and have their blood pressure and heart rate monitored by implantation of radio-telemeters. Cardiac hypertrophy will be induced by aortic banding (a procedure that involves reduction of the aorta diameter by ligation to simulate chronic increase in blood pressure) or by administration of isoproterenol via an osmotic minipump (essentially a small capsule) implanted subcutaneously, a treatment that induces an increase in heart size and remodelling. The progression of the disease will be closely monitored by non-invasive techniques and invasive cardiac investigations as terminal procedures. Expected side effects of the procedures proposed are respiratory problems, hypothermia, dehydration and thoracic pain of moderate severity. At the end of the procedure the animals will be killed humanely.

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Deciphering Neural Crest Gene Regulatory Network</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Neural crest, zebrafish, gene regulatory circuitry</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Embryo development is driven by a large set of finely choreographed gene regulatory programmes that control all aspects of cell behaviour, ultimately leading to the formation of a complex multicellular organism. Understanding how these programmes, encoded at the genome level, are translated into intricate networks of interacting biological components (genes, RNA and proteins) is essential to our understanding of developmental mechanisms within a cell and thus in turn provide insight into human diseases that arise when biological processes go awry. Here we aim to decipher the regulatory programme that orchestrates the early steps of neural crest formation. The neural crest (NC) is an embryonic cell population, similar to stem cells, in that they are ‘multipotent’ and give rise to a number of different cell types and tissues to form a vertebrate body. Those include but are not limited to sensory neurons (mediating the sensations such as touch, pain, temperature etc.), smooth muscle of major blood vessels and heart valves, craniofacial skeleton and cartilage forming the majority of our skull including the jaw, and the vast majority of the body’s pigmentation. The main objectives of this project are:
1. Define the details of the regulatory programme that governs the formation of neural crest cells during the pre-migratory stage. During this critical period neural crest cells start the EMT program (Epithelial to Mesenchymal Transition), establish their multipotency and convert to migratory</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

	phenotype. 2. Understand how the remodelling of chromatin contributes to this complex network and dynamic changes to it. 3. Identify the genomic regions (hot spots), which when altered result in prevalent neural crest-related birth anomalies.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our main objective is to construct a hierarchical representation of the network of genes that control the start of a cellular process called epithelial to mesenchymal transition (EMT). During EMT, epithelial cells are converted from a tightly attached sheet of cells into a more dispersed (mesenchymal) cells. EMT occurs reiteratively during development and is critical for events ranging from formation of germ layers to the detachment of specialized cells that build different organs. Importantly, the abnormal appearance of EMT process in the adult precedes tumour progression and is a hallmark of tumour metastasis. EMT has previously been studied from a cellular perspective in *in vitro* conditions, but the gene regulatory regulatory circuits controlling EMT onset *in vivo* have not yet been characterized. This study aims to characterize this process in the neural crest, which is a “paradigm” for the process of EMT and as such provide connections between embryonic EMT and metastatic cancer EMT. Understanding how is this process initiated and what regulatory interactions control it, would allow us to better define possible therapeutic interventions and prevent metastasis. Hence obtaining a holistic picture of regulatory control of embryonic EMT could help prevent abnormal EMT that cancer cells undergo just before they becoming invasive. Neural crest-related anomalies, such as craniofacial and some heart malformations, account for ~1/3 of congenital birth defects. These occur when genes or gene regulatory circuits are disrupted during embryonic development. Our approach thus offers the means of understanding the mechanisms underlying neural crest-related birth defects and provides the perspective of correcting /attenuating such anomalies. Once fully resolved, the neural crest gene regulatory information could be used in directed differentiation assays of stem cells into selected

	specific neural crest derivatives for regenerative medicine and stem cell therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	We intend to use zebrafish *Danio rerio*, a well-established developmental model organism. We expect to raise about 30,000 animals during the period of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will use zebrafish transgenic animals to obtain embryos for our study. The level of severity is mild, as the transgenes are not detrimental to the health of fish and do not alter normal development. The embryos are obtained by natural spanning. Animals will be humanely killed at the end of their reproductive age.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The in vitro models of EMT cannot be used, as those cells are either artificially induced to change shape or already in late stages of the transition. This study aims at providing a natural regulatory picture using in vivo context of the developing embryo using model organisms. Neural crest gene regulatory circuits are highly conserved amongst vertebrates, and information obtained in our study will be applicable to human health. Our approach uses vertebrate embryos that have a stereotypical EMT, but develop outside the mother, and their generation and subsequent analysis requires minimal number of adults. In parallel to zebrafish, our laboratory uses early chicken embryos to study static aspects neural crest gene regulatory network. Chicken embryo is an alternative to the mouse, and has the advantages of the mammalian system (conserved genome, similar mode of development to human), but is also highly accessible to manipulation and embryos are obtained without sacrificing the mother. For studying dynamic changes to the network, as well as address mechanisms underlying neural crest-related birth defects, we need a genetically tractable organism and propose to use the zebrafish. Due to their high fecundity, external embryonic development, ease of transgenesis and genome editing, zebrafish is highly amenable to high-throughput genomic studies proposed here.
2. Reduction Explain how you will assure the use of minimum numbers	Nature of project is such that systems level analyses are performed in small number of cells, thus minimising the number of embryos

of animals	necessary for each assay. Embryos are produced by natural spawning of transgenic zebrafish lines in continuous use hence minimal number of adults will be used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The experimental analysis on zebrafish embryos is performed at early stages of neural crest development. Given that neural crest itself forms sensory system and at the time of collection those cells are still only precursors, the embryos used have no neurophysiological sensitivity, given that the neural crest-derived sensory system is still not formed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Development of an allogeneic aortic graft</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Tissue Engineering, vascular bypass graft</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Two</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td>~~Yes~~</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The project aims to develop novel large diameter biological arterials grafts, which can be used to repair large arteries following disease or injury and to replace synthetic grafts that became infected following surgery. Vascular graft infection is a rare but severe complication of arterial repair or bypass, the mortality rates for graft infection can be as high as 50 % even with aggressive therapy. Biological grafts are more resistant to infection than those made from synthetic materials, however currently available biological grafts is in short supply and associated with many complications. Decellularisation of human arteries will allow for an off the shelf therapy which will provide a lifelong alternative treatment following graft infection or for primary bypass or artery repair. Decellularisation is a method to remove all the cells and immunogenic material from tissues, this results in a scaffold which retains its mechanical strength and is composed of natural proteins which allows regeneration by the patients own cells following implantation.

This section of the project aims to evaluate the cellular and immune response to the graft as well as the mechanical function of the graft once implanted into a large animal.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td>• The study will test how this type of arterial graft heals and determine if they are able to function as an artery over a short implantation time.
• If successful the work may be allowed to</td></tr>
</table>

[5] Delete Yes or No as appropriate.
[6] At least one additional purpose must be selected with this option.

project)?	continue to clinical trails of decellularised medium and large diameter human arteries for the replacement of infected grafts. • Improvement in the mortality rates of primary synthetic graft infection. • Reduction in the number of reoperations required as a result of graft infection and a reduction in hospital recovery time.
What species and approximate numbers of animals do you expect to use over what period of time?	Large White Pigs, eight animals over two years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Surgical procedures will be performed under stable general anaesthesia using aseptic techniques. Induction and maintenance of anaesthesia will be carried out but the veterinary surgeon with vast experience in pig anaesthesia. Transplantation of acellular arteries will be carried out of by an experienced vascular surgeon who has previous experience of *in vivo* work in sheep and pigs. Blood loss is not expected. However, if any leakage of blood at the site is observed, vascular sealants will be applied. Similarly post-operative haemorrhage is not expected. General complications of abdominal surgery in pigs include wound infection (< 2 %), wound breakdown (< 1 %) or herniation (2 %). Wound infection will be treated with antibiotics. If the wound breaks down, and is not infected, it will be re-closed under general anaesthesia. An abdominal dressing will be applied for two to five days to reduce the incidence of herniation. Herniation in the absence of any wound infection or ulceration and not causing any discomfort to the animal will be left untreated. However, moderate herniation may warrant re-suturing of abdominal incision under general anaesthesia and under the instructions of Designated Veterinarian. This will only be done once in post-operative period. If hernia develops for the second time, animal will be humanely killed. Animals will receive regular health checks at least twice a day for the first five days post-operatively and subsequently will be inspected daily. Post-operative pain relief and antibiotic cover will be given routinely and depending upon the condition of the animal. Animals will be sacrificed if serious complications are suspected and/or the animal shows persistent distress and cannot be treated. Removal of small amount of blood sample from

	superficial vein is not expected to cause any adverse effect.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no alternative to the use of animals for the pre-clinical testing of arterial grafts prior to moving to clinical trails o human subjects. A large battery of laboratory tests have be preformed however these were unable to provide the data necessary to provide the confidence to progress to clinical trails. Large animal models are they only method available to evaluate the cellular reaction and host response to these grafts. Safety testing has been carried out in the lab using human and animal cells however these are unable to replicate an animal model, however it has been determined that the material will not be toxic or cause any direct harm to the animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals utilised in the transplant protocols has been reduced by including the minimum number of replicates and all subsequent *in vitro* analysis will be performed on these replicates negating the need for additional animals. This is the minimum number of replicates that will allow for loss of animals die to unrelated causes and still leave sufficient numbers for statistical analysis. Previous work within the research group has established a good correlation between *in vitro* biocompatibility and *in vivo* biocompatibility in a small rodent model. Thereby negating the need to repeat a small rodent model for the current study.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	All procedures are carried out under general anaesthetic and pain relief will be routinely administered as required. Whilst major surgery the implantation of the graft material will carried out by a qualified surgeon under the supervision of the named veterinary surgeon. Patient monitoring systems (ECG, blood pressure, pulse oximetery), supplementary heat and vascular expanders will be employed routinely. Any side effects have hopefully been predicted and we have protocols in place to adequately deal with these whilst causing the animal as little pain and suffering as possible.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Evaluation of Toxic Hazards to Fish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ecotoxicology, freshwater, marine, fish</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>Five years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[7]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To generate quality-assured data on ecotoxicological properties of anthropogenic substances for clients as part of the evidence they present to regulatory authorities for assessment of the risks to the environment posed by those substances when they are produced, transported or used. The data required will normally include studies on effects on representatives of the major trophic levels in the environment including bacteria, plants and/or algae, invertebrates and fish.

These data, in addition to data for environmental fate and exposure, will be used to assess the level of environmental risk associated with those substances.

The studies with fish, which is the purpose of this licence, constitute an important and mandatory part of the risk assessment process.

A number of study types or protocols involving fish may be required depending on the level of production and fate and potential risk of the substance.

These include:

- Acute toxicity to fish (96-hour LC50 test or limit test) e.g OECD203.
- Prolonged toxicity to fish over 14 days e.g. OECD204</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

	- Fish growth study over 28 days e.g. OECD215 - Bioconcentration in fish e.g. OECD305 - Effects on fish early life stages e.g. OECD210
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	To assure environmental safety of substances by assessing the intrinsic ecotoxicological hazards of chemicals, products or effluents. The environmental risk will be assessed by a regulatory authority and where unacceptable risk to the environment is identified the production and use of the substance will be restricted or banned. Appropriate labelling and risk phrases on packaging for products on general sale will be applied according to the results of environmental fate and ecotoxicology studies. This will allow appropriate precautions to be taken when using and disposing of those chemicals to minimize risk to the environment
What species and approximate numbers of animals do you expect to use over what period of time?	The species used will be one of the following: Rainbow trout (*Oncorhynchus mykiss*) Carp (*Cyprinus carpio*) Zebra fish (*Brachydanio rerio*) Turbot *(Scophthalmus maximus*) Sheepshead minnow (*Cyprinodon variegates*)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mortality and other sublethal effects such as loss of equilibrium, abnormal swimming or unresponsive to external stimuli. Levels of severity will be moderate or severe. Fish will be killed by a Schedule 1 method at termination of a test (AC).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is limited scope for replacement as the acute fish test is a regulatory requirement under many regulatory submissions
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction of numbers of fish used is achieved in three ways; 1) by always using the minimum number of fish allowed by the guideline to generate statistically valid data, 2) by carrying out limit tests (tests using a single concentration) using toxicity results previously generated from algae and invertebrate tests to define the test concentration (e.g. the use of the OECD Threshold Approach), 3) by using toxicity results previously generated e.g. using the results from a freshwater test for registration of an offshore chemical or the use of data generated from older studies that were not

	GLP-compliant.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Refinement is achieved by performing careful visual inspections of the fish under test in order to minimise their suffering. For example moribund fish (fish showing no response to external stimuli and /or little respiratory activity) will be removed and humanely killed.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Neuronal function and psychiatric disorders</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Autism, schizophrenia, neuronal activity, plasticity.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[10]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Sufferers of neuropsychiatric diseases frequently have disruptions in their genes. We are beginning to discover which genes these are, but we currently lack understanding of how disruptions to these genes result in abnormal behaviours. In particular, we do not know how the complex functions of activity in the brain are altered during behavioural tasks (such as social interaction) in conditions like autism. This project aims to address these questions by looking at how genetic disruptions liked to neuropsychiatric diseases affect brain function and behaviour.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The potential benefits of this project are two-fold:
(i) Scientific knowledge will be advanced by the results of this project. As stated, there is a lack of understanding regarding how genetic disruption can cause psychiatric disorders. This project will deliver new findings across multiple levels to inform the scientific community about new avenues to research.
(ii) Humans can benefit in the longer term by the insights delivered from this work. Drugs that improve the symptoms of psychiatric diseases are of limited success and can have some terrible side-effects. This Project plans to better understand the basic mechanisms of how neurons function in models of psychiatric diseases, thereby aiding the development of more effective drugs.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>This Project will use predominantly mice but also rats, both of whom will have genetic alterations relating to psychiatric disorders.</td></tr>
</table>

[9] Delete Yes or No as appropriate.
[10] At least one additional purpose must be selected with this option.

over what period of time?	It is predicted that no more than 2000 mice and 500 rats will be used within the Protocols of this Project over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	No Procedure is expected to exceed a 'Moderate' severity rating. Behavioural testing will be performed as a battery (i.e. each animal will be subjected to several different experiments), but limits will be made as to how many (total) and the number of experiments with aversive stimuli that any one animal will receive. The administration of certain drug to modify behaviour will be carefully monitored for any unexpected reactions and the choice of drug will be checked with the Vet and its function in rodents examined through previous published studies. The surgery to implant electrodes or mini-osmotic pumps is well established and should not cause any lasting pain or distress. However, to improve post-operative outcomes, appropriate anaesthesia and analgesia will be selected with consultation with the Vet. Careful post-operative monitoring will be made to ensure no suffering. At the end of the procedures, animals will be killed by either Schedule 1 or a non-schedule 1 method (perfusion) or if appropriate, the animals will be used for further breeding.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project aims to understand how genetic abnormalities related to human psychiatric disorders alters brain structure and function. In particularly, we will focus on behavioural outcomes and neural processing. Unfortunately it is not possible to perform these experiments in humans; the tissue is not available and it is not ethically acceptable to implant scientific apparatus in human brains for extended periods. Computer models also cannot be used. There is a general lack of information to make models that can sufficiently guess at how the brain functions, especially considering that this project will look at genes for which there is little published information.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure reduction writing a protocol for each experiment that will include statistically designed group sizes (by power calculations) and searching the literature to ensure that experiments are not unnecessarily duplicated. Breeding protocols will also be designed to ensure that only the required amount of animals are bred to minimise wastage. Within this project, we will be using an advanced type of neural recording that will provide us with

	multiple sources of data while the animal is performing a behavioural task. This will mean that we can ask many more questions from our recorded data, and how they related to an awake, behaving task, than conventional studies examining these aspects in isolation. This approach will mean that we can reduce the number of animals that we would have otherwise have used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will be using predominantly mice but also rats within this project. Rats and mice share a very similar genetic makeup to humans, and their neuroanatomy is also similar. There are also many behavioural tests that allow us as scientists to assess psychiatric disorder-like behaviours in rodents, something that cannot be done in lower model systems. We are undertaking a series of experiments that have been shown by others not to case suffering. Before all experiments, the rodents will be handled to reduce the stress of human interactions. Behavioural experiments in general do not case pain and suffering, but suitable gaps between experiments will be given and there will be a limit on the use of aversive stimuli given to any one animal. For surgical procedures, suitable anaesthesia and analgesia will be administered in discussions with the Vet, and any sign of suffering will be taken to the relevant people for immediate advice.