Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2013

Volume 54

1. **Environmental regulation of fish physiology**
    - Fish, environment, reproduction, stress, growth

2. **Mechanisms of cortical oscillations in health and disease**
    - Neuron, oscillation, neocortex, neurology, psychiatry

3. **Effect of shift-work on adipose circadian rhythms**
    - Circadian, sleep, shift-work, desynchrony, metabolism

4. **Environmental sensitivity of subclinical disease resistance and resilience in farm animals**
    - Environmental, sensitivity, disease, farm animals

5. **Safety Testing Using Small Animal Species**
    - Regulatory Safety Assessment Small Animal

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    - Safety Assessment Studies Method Development

7. **Interaction between dell cycle regulation, development, and diseases**
    - Cell cycle, development, human diseases, cancer, retinal disorders

8. **To study the epidemiology and pathogenesis of the diseases**
    - Virus, salmonid, IHN, VHS, aquaculture

9. **Glucocerebrosidase Deficit in Parkinson's Disease**
    - Glucocerebrosidase, Gbal, Parkinson's disease, alpha-synuclein

10. **Regulation of muscle stem cell function and metabolism**
    - Adult stem cells; muscle; regeneration; ageing; metabolism

11. **Induction and resolution of renal inflammation**
    - Kidney, inflammation, autoimmunity, vasculitis, leukocytes

12. **Regulation of T cell immunity and autoimmunity**
    - diabetes, autoimmunity, T-cells, immune regulation

13. **Control of excitable cell (nerve/muscle) function**
    - control of excitable cell function

14. **Neurobiology of sensory processing**
    - Sensory processing, feedback, attention

15. **Investigation of the Role of Nox and Reactive Oxygen Species in the Pathogenesis of Metabolic Disorders**
    - Cardiovascular diseases; oxidative stress; type 2 diabetes; NADPH oxidase

16. **Lung repair and regeneration**
    - Injury, repair, regeneration, cell fate.

17. **Skeletal phenotypes in health and disease**
    - Skeleton, osteoclasts, osteoblasts, honnonal, stress

18. **Epithelial regeneration and carcinogenesis**
    - Lung, thymus, regeneration, cancer, repair

19. **Transcription factors in development And disease**
    - Transcription factor, gene expression, development, disease, normal cell death

20. **Development of healthy oocytes and embryos in mammals**
    - Fertility, eggs, embryos, chromosome-segregation, early development

21. **Biology of normal and leukaemic cells**
    - Leukaemia, haematopoiesis, therapeutic target, disease modelling

22. **Eye defects, development and repair**
    - Eye development, congenital, stem cell

23. **Improving light-activated therapies**
    - PDT, PCI, Photosensitiser, Chemotherapeutic, Laser

24. **Coding sound in the normal and hearing impaired nervous system**
    - Hearing-impairment, cochlear implants, pitch, distortion

25. **Injury and regeneration in the nervous system**
    - Multiple sclerosis, regeneration, stem cells

26. **Discovery ADMET studies for novel therapeutics**
    - ADMET, pharmacokinetics, discovery, DMPK, preclinical

27. **Development and plasticity of synapses and networks**
    - Nerve cell, synapse, development, energy, intracellular transport

28. **Toxicity Testing II**
    - Toxicology, rodent, medicine, appearance, histology

29. **FMOs: exogenous and endogenous metabolism**
    - Trimethylaminuria; drug therapy; metabolism

30. **Information processing in mammalian brain circuits**
    - neuron dendrite synapse sensory rodent

31. **External Fish Parasites and Therapeutics**
    - Aquaculture, parasite, fish

32. **Breeding and maintenance of genetically altered mice for central nervous system regeneration research**
    - Multiple sclerosis, regeneration, stem cell

33. **Assessing the feasibility of using an animal model for in vivo taste assessment of pharmaceutical compounds and formulations**
    - taste assessment, lickometer, rodents, APIs, formulations

34. **Safety and efficacy of veterinary medicines**
    - Veterinary medicines safety efficacy testing

PROJECT 1	**Environmental regulation of fish physiology**	
Key Words (max. 5 words)	Fish, environment, reproduction, stress, growth	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	y	Basic research
	y	Translational and applied research
	n	Regulatory use and routine production
	n	Protection of the natural environment in the interests of the health or welfare of humans or animals
	n	Preservation of species
	n	Higher education or training
	n	Forensic enquiries
	n	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objectives of the project are *1)* to better understand the entrainment and regulation of reproduction, growth and development by environment factors (e.g. light, temperature, salinity and nutrition) in fish (Environment and physiology), 2) To study stress and behavioural responses of fish to alterations of the environment (e.g. light, temperature, salinity, sound, water quality) (Chronobiology and stress/behaviour) and 3) To study the impact of genetic manipulations (selection, ploidy) and hormonal sex control on fish performance.	
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 basic understanding of fish reproduction acquired by the bA reproduction group led to the development of protocols for the fish farming industry to control early maturation, optimise productivity and fish welfare, and protect wild stocks. Reproduction in farmed fish stocks results in the reallocation of energy from somatic to gonadal growth leading to reduced flesh quality and muscle growth, increased susceptibility to disease and welfare concerns. All of these lead to production losses through mortality and product downgrading during	

	processing. Key strategies have been identified and researched to tackle this problem e.g. reduction in the prevalence of early maturing fish prior to harvest either using photoperiodic treatment, sterility using chromosome set manipulation or monosexing. The knowledge gained by the group in these areas has led to the implementation of protocols, guidelines and practices within the industry that have significantly improved the sustainability of the sector, generating growth and increased profitability. In addition, the expected benefits of the work carried out under this project includes refined husbandry protocols and environmental tolerance through stress studies and programming at an early stage of development (nutritional programming and epigenetic effects) to optimise fish welfare and performances from larvae to adult broodstock in established and new candidate species.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will focus on fish species of interest to aquaculture (e.g. Atlantic salmon, Atlantic cod, sea bass, tilapia...) and model species for more fundamental studies (e.g. zebrafish). Over the full duration of the PPL, it is expected that <30,000 fish will be used per year (juvenile to adult fish) and <60,000 larvae from first feeding to weaning.
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 project involves procedures which are all categorised as 'mild' and good handling techniques and appropriate anaesthesia will be used throughout. Few adverse effects are expected and fish will be closely monitored after experimental procedures are performed. The staff that will be involved in the studies are experienced in fish care, and have all undertaken suitable training. Most of the protocols described here involve techniques that are routinely used in broodstock management (alteration of rearing temperature or photoperiod or salinity, anaesthesia, length-weight measuring, stripping, ultrasound...). Fish which have been subjected only to such techniques may be released to stock or to commercial stocks as these procedures are mild and do not warrant the unnecessary killing of fish. This would not apply if fish have been subjected to manipulation of endocrine status which would be killed by a schedule 1

	method as part of the protocol.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mechanisms we are interested in are **a** product of the interaction of various specialised neuroendocrine tissue structures and there exists no isolated cell based model that can recreate such an interaction in the target species of interest, thus the potential for replacement of animal experimentation is limited. However, while the majority of work requires animal based experimentation, isolated discrete tissues (e.g. primary cell culture) will be utilised. Where possible, non-invasive monitoring of fish behaviour and environmental stimuli will be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers used in all studies are defined by power analysis to set an effective sample size. Where non-terminal sampling is performed the minimum sample size is taken as required for the planned analysis. Where terminal sampling is performed and tissue samples harvested for subsequent analysis, excess samples will be archived for future potential use to reduce the requirement for any subsequent experimentation.
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.	Work will be carried out on commercially important Teleosts plus zebrafish. Given the diversity of species, farming systems and environments in aquaculture it is essential to target a broad range of species for research purposes to ensure project objectives are attained for the desired species. Zebrafish is a common and useful model organism for studies of vertebrate development and gene function. Where possible, zebrafish will be substituted for other target species. Throughout the work all animals will be housed in conditions that comply with welfare standards of the target species of interest and monitored by the NACWOs.

PROJECT 2

Mechanisms of cortical oscillations in health and disease

KEYWORDS

Neuron, oscillation, neocortex, neurology, psychiatry

This project aims to understand how organised brain electrical activity is generated and how this activity can go awry in neuro-psychiatric disease.

Our current understanding of how the brain works is based on recordings of electrical activity that is measured using electroencephalogram (EEG) in humans. These patterns of electrical activity can change over time and across different parts of the brain and these alterations lead to a response. Examples range from our ability to express emotions to the ability to move our limbs in a controlled manner.

The main limitation of current approaches is the lack of knowledge of how neurons in the brain produce electrical activity patterns that underlie the BEG. To do this would mean inserting electrodes into the human brain. This is, in the majority of cases, ethically unviable.

In order to gather useful and meaningful data about this question, brain tissue will be obtained from animals in which their normal brain function is similar to humans and animals that have transgenic or chronic experimental modifications that mimic the core symptoms associated with schizophrenia and epilepsy. Reductions in animal use will be achieved by the use of human tissue and the construction of computational models of large-scale brain function.

Epilepsy will be modelled by an administration of an agent to provoke the development of epilepsy over a period of time (in some cases animals will be kept for up to one year). This results in a chronic epileptic model in an animal. To model schizophrenia we will adopt two approaches. Firstly, we will use genetically modified mice. Genes that confer risk in schizophrenia have been altered in these mice. We aim to examine the impact this manipulation and examine the downstream effects for brain activity. Secondly, animals will undergo an environmental stress early in life. Animals will be removed from their mothers for either a 24-hour period or for repeated brief periods of time during a time window critical for brain development. Multiple assessments of these manipulations, in the form of assays of behaviour, brain electrical activity and the expression of specific neurons will be conducted. This will maximise the amount of information gained from the animals used.

For the duration of the study we will need to use 1650 rats and 850 mice. We use rats and mice as they have been shown to be a good model of basic brain function. Specifically, electrical activity observed in the EEG can be reliably

replicated. Using sections of brain taken from the animals following deep anaesthesia and being humanely killed. All data gathered is then translated into a computational model that will aid in the long-term reduction and refinement of future experimental work.

The immediate benefits of this project will be an understanding of mechanistic features of organised brain activity. Within this context we will provide information on the basis of neurological and psychiatric illnesses at the level of the neuronal microcircuit. This will aid in our interpretation of these signals in brain function in the clinical setting. Long-term these details may aid in the development of therapeutic approaches.

PROJECT 3

Effect of shift-work on adipose circadian rhythms

KEYWORDS

Circadian, sleep, shift-work, desynchrony, metabolism

Body clocks driving daily rhythms are important for synchronising physiological and behavioural activity with predictable changes in the internal and external environment such as light-dark cycles and daily meals. Body clocks are found in almost every tissue of the body. The main clock in the brain is synchronised with the light-dark cycles, while clocks in tissue such as liver and fat are mostly synchronised to meal timing.

During shift work, we desynchronise the timing of sleeping and eating from the light-dark cycle. It is thought that, this mistiming causes some of the negative health effects of shift work such as overweight and increased risk of developing diabetes. Because this mistiming of sleep and feeding may change the timing of body clocks in fat tissue, but not in the brain, it is thought that this is what is actually happening, be we don't know exactly how this occurs, and what this implies for the daily rhythms in the physiology of fat tissue.

This project explores three different pathways that may underlie the dysregulation of daily rhythms in fat tissue during shift —work:
1. Altered timing of feeding.
2. Altered expression of genes that affect both body clocks and physiology in fat tissue.
3. Altered timing of heat generation by fat tissue during sleep.

We will place mice, rats and voles on a shift work routine. During shift work, we will measure sleep brain waves, body temperature, feeding activity and consumption and compare changes in those measures with alterations of daily rhythms in fat tissue. We use wireless technology for the measurement of sleep brain waves, body temperature and behavioural activity, and this mininlises the suffering of the animals during the shift work pattern.

While mice and rats are chosen because much is known about their biology, we also choose to use voles. Voles do not show clear daily rhythms in feeding and activity, and are just-as active in the light and the dark. When we compare these animals to mice and rats, we can uniquely see the impact of daily rhythms. This approach is much more refined than supressing circadian rhythms in mice and rats.

The circadian timing mechanism consists of many clocks and rhythms that all interact. Much of these interactions between these systems are comprehensive and for a large part unknown. Therefore we need study these effects of mistimed sleep in whole organisms, where the circadian timing mechanism is intact. Because we need

to collect tissue, we cannot use humans and therefore we require these animal models.

We know that 15% of the UK working population regularly works shift, and it is known that there shift workers are more likely to develop unhealthy weight gain and diabetes. While we know that fat tissue is a key tissue in developing these health problems, we know almost nothing about what happens to this tissue. This research aims at understanding the involved mechanism, with an emphasis on mistiming of body clocks and daily rhythms.

PROJECT 4

Environmental sensitivity of subclinical disease resistance and resilience in farm animals

KEYWORDS

Environmental, sensitivity, disease, farm animals

• **Summary**
This program of work assesses nutritional, genetic and environmental sensitivities of (the outcome of) sub-clinical disease in farm animals.

• Rational
Drug resistance hampers disease control and we need to know whether alternative nutritional strategies depend on other factors. Feeding extra protein to sheep reduces worms but may be breed dependant. Feeding probiotics to young pigs improves gut health but may depend on diet composition and breed. Many plants may have anti-parasitic properties, and we aim to identifS' their efficacy. We also assess if climate change affect parasitism in sheep. Results will inform strategies in farm animals to achieve disease control with minimal use of drugs and better predict future disease risk from climate change.

• Outline
Sheep will be infected with worms or left uninfected, and then dosed or fed with plant extracts, or fed different levels and types of protein. Sheep will be housed at different environmental temperatures and infected with parasites reared under different climatic conditions. Pigs will be infected with bacteria and fed probiotic containing foods with different nutritional compositions.

• Animals use
Complexity of underlying interactions between digestive, immune and endocrine functions requires animal use, although *in vitro* studies will pre-test anti-parasitic plants.

• Suffering Our refined infection and nutrition protocols cause little or no harm, and experimental foods have high quality ingredients. Animals deliberately fed a little bit below their nutrient requirements simply grow slightly less or produce slightly less milk without suffering. Animals are daily observed, and quantifiable, clear end-points have been established to ensure that animals do not exceed a mild to moderate severity limit.

• Why sheep and pigs
This program of work uses sheep and pigs both as model and target animals. Although many aspects of nutritional sensitivity of parasitism are being addressed in rodent models, demonstrating breed effects and underlying host responses in target animals remain required to predict impact of alternative disease control strategies.

Furthermore, there are no suitable alternative rodent models to predict nutritional sensitivity of gut health for weaned pigs.

• Animal numbers, procedure description, adverse effects

We may use up to 140 sheep and 160 pigs per year, which we minimise through lab-based studies and through statistical tests to identify the minimal number required to observe desired effects. Nutritional and infection protocols are used to study effects of 1) protein nutrition on worm control and immune responses in sheep, 2) anti-parasitic plants on worm infections in lambs, 3) environmental temperature and humidity on parasitism in sheep, and 4) probiotics on gut health in weaned pigs. Protocols are sufficiently refined to only expect mild to moderate adverse effects.

• Benefits

We aim to learn how plants with anti-parasitic properties, protein nutrition, probiotics and climatic influences influence (the outcome of) sub-clinical disease in farm animals. This may better predict impact of climate change on disease risk and inform feeding strategies to achieve disease control with minimal drug use. This would benefit both conventional and organic farmers, but also people in developing countries, where infection and malnutrition often go hand in hand.

PROJECT 5	**Safety Testing Using Small Animal Species**	
Key Words (max. 5 words)	Regulatory Safety Assessment Small Animal	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)		Basic research
		Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Humans are exposed to xenobiotic materials as patients, consumers and workers. In order to allow sound regulatory decisions regarding safe human exposure levels to xenobiotics, it is essential to conduct a risk assessment by relating the intrinsic hazard profile of the material to the desired or likely exposure in man. This project licence authorises the conduct of in-vivo studies in laboratory small animal species to evaluate the hazard profile of xenobiotics in terms of general toxicity, oncogen icity and toxicokinetics.	
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 principal benefit of this project is the provision of safety data to facilitate sound regulatory decisions regarding human exposure to xenobiotics.	

What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year life of this Project Licence, it is estimated that 47,350 mice, 67,200 rats, 5,820 hamsters, 760 guinea pigs and *2,650* rabbits will be used.
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 majority of animals on shorter term studies are expected to have mild adverse effects such as slight weight loss or changes in appearance or behaviour. A small number of animals (usually limited to the highest doses evaluated in early studies) may show more significant adverse effects. Humane endpoints will be adopted or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively less adverse effects. The majority of animals will be humanely killed at the end of a study; investigations may include sampling of various organs and tissues followed by microscopy to evaluate potential changes, and detailed examination of parents, foetuses and offspring.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present there are no scientific and legally acceptable evaluations of systemic toxicity that will satisfy regulatory requirements other than use of animals, though validated *in vitro* tests for specific organs are used wherever possible. Where available, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology information will be utilised to reduce animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies are designed to provide maximal scientific value from the minimum number of animals, whilst using sufficient animals to meet scientific objectives, and regulatory guidelines. Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of studies. Where available, sensitive analytical techniques (eg Dried Blood Spot analysis) may be used to reduce animal numbers. Wherever practicable, the combination of endpoints eg general toxicity, reproduction and developmental toxicity, safety pharmacology, mutagenicity etc in studies is

	considered, to reduce overall animal usage.
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.	Species choice and use of specific animal models is determined by the need to generate regulatorily-acceptable data. Where a choice of species is possible, care is taken to select the most biologically appropriate species, and the species which most closely relates to man. Animal welfare costs are minimised by the careful selection of dose levels to reduce the likelihood of unexpected toxicity, and the application of rigorous and comprehensive humane endpoints. Individual studies are designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinary support, purpose built facilities and a clear focus on animal welfare.

PROJECT 6	**Safety Assessment Studies - Method Development and Validation**
KEYWORDS	Safety Assessment Studies Method Development

Summarise your project (1-2 sentences)
This Project Licence authorises the conduct of *in-vivo* studies in laboratory animal species to allow new tests and test methods to be developed and validated for use. The new tests and test methods can then be implemented on existing safety assessment Project Licences at this facility.

Outline the general project plan.
Studies conducted on this Project Licence allow the development and validation of new tests, and new test methods, for use in the non-clinical regulatory assessment of general toxicity, oncogenicity, and developmental and reproductive toxicity. Such work includes the feasibility of using novel dosing methods, validation of new biomarkers, use of new technology, refinement of current methods, and generation of background data to assist with study interpretation, including testing the sensitivity of methodology by the use of reference materials.

Studies may assess effects upon adults, parents, the developing foetus, and offspring. Animals are observed regularly in order to monitor changes in appearance and behaviour. Data may be collected on food and water consumption, bodyweight, health status, the functional status of biological systems, and developmental and reproductive performance. Samples of body fluids, typically blood and urine, may be taken for analysis at intervals defined carefully to minimise adverse health effects. Depending upon the objective of the study, at the end of a study animals may be retained at the laboratory, or humanely killed; post-life investigations may include sampling of various organs and tissues followed by microscopy to evaluate potential changes, and/or detailed examination of foetuses.

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.
Humans are exposed to xenohiotic materials as patients, consumers and workers. In order to allow sound regulatory decisions regarding safe human exposure levels to xenobiotics, it is essential to conduct a risk assessment by relating the intrinsic hazard profile of the material to the desired or likely exposure in man. The hazard profile is normally established via the conduct of safety assessment studies in animals. The design of such studies is driven by regulatory authorities; new tests, new test methodology and new technology require development, validation and implementation in order to meet changing regulatory requirements, and to provide improved data for regulatory decision-making.

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 majority of animals on this Licence are expected to show, at most, mild adverse effects such as slight weight loss or changes in appearance or behaviour; many animals will show no overt adverse effect at all. A small number of animals (limited to studies where there is a requirement to demonstrate some toxicity) may show moderate adverse effects. Humane endpoints will be adopted or dose levels reduced if animals show excessive effects. Longer term studies are expected to have progressively less adverse effects.

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

The benefits of this project are that new tests, and new or improved methodology, will be introduced into the testing strategy for safety assessment of novel xenobiotics. In turn, this will provide regulatory authorities with improved information to assess the risks to which humans are exposed when such substances are produced, transported or used.

A notable potential benefit of the project is reduction and refinement of animal usage, through (for example) the validation of methodology which has beneficial animal welfare outcomes such as reduced stress levels, or which produces better quality data with lower variability. This, in turn, may allow a reduction in the numbers animals used to achieve the same outcome.

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,

Over the life of this Project Licence, it is estimated that 1320 mice, 840 rats, 180 hamsters, 180 rabbits, 36 dogs, 50 minipigs and 48 non-human primates will be used per year. Species selection will be based on regulatory requirement. The minimum number of animals will be used by following good scientific practice, referral to published guidelines on study design, and statistical analysis. An in-house programme of ethical review will advise the Project Licence Holder on the 3Rs (Replacement, Reduction and Refinement).

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.

Alternatives to in vivo animal models for safety assessment are being developed and used where possible. However, there are currently no alternatives available for evaluation of general toxicity, oncogenicity, and developmental and reproduction toxicity that are acceptable to regulatoty authorities for the purpose of assuring safe use of, or exposure to, a xenobiotic in man. It is therefore necessary to make this evaluation in whole body systems (live animals). -
In order to refine and extend the use of safety assessment studies using animals, there is no alternative to using animals for developing and optimising methods, and validating them. Where available and relevant, review of scientific articles, non-animal methods and other animal data such as metabolism and pharmacology

information will be utilised to reduce animal use.
Explain why the protocols and the way they are carried out should involve the least suffering. Individual studies will be designed to cause the least possible suffering by frequent review of practices, provision of highly skilled technical staff and veterinaly support, purpose built facilities and a cleat' focus on animal welfare.

PROJECT 7	**Interaction between dell cycle regulation, development, and diseases**	
Key Words (max. 5 words)	Cell cycle, development, human diseases, cancer, retinal disorders	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Many human diseases results from defect of cell cycle regulation, development, and their interaction. However, little is known about the mechanisms of their interaction. Also, it is largely unknown about the molecular mechanisms, which bring to human diseases. This project will investigate the mechanisms, aiming to understand human diseases such as cancer and eye disorders.	
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)?	This will elucidate molecular pathology of human disease such as cancer and eye disorders. This understanding is essential for development of new diagnosis and treatments. This project will try to develop novel diagnosis of cancer and eye disorders. This project aim will try to develop novel treatment of cancer and eye disorders.	

What species and approximate numbers of animals do you expect to use over what period of time?	Xenopus: 400 frogs in five years Mouse: 5,000 mice on five 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?	Xenopus: we will maintain the animal to obtain eggs and testis. Eggs will be obtained by hormone injection. All experiments are at mild level. Mouse: we will maintain genetically modified mice. In order to develop new treatment, we will use some chemicals, virus, or cells into the mice. The severity
Application of the 3Rs	level is mild or moderate.
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use in vitro cell culture system. However, this work is mostly complex developmental biology, with multiple interacting tissues. Processes of embryogenesis or retinogenesis cannot generally be reproduced by cells in culture, so we have to perform studies on whole embryos. Also, this project aims to evaluate roles of genes that regulate co-ordination between cell cycle regulation, determination, and differentiation in systematic human diseases. Although we use in vitro systems, such in vitro systems are not sufficient to evaluate the roles in human diseases. Therefore, combination of in vitro and in vivo system is essential.
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	Through combination of Xenopus and mice, we will reduce the number of higher organism, mouse. Also, we will re-use the female Xenopus to obtain eggs, which significantly reduce the number of Xenopus. Also, we will design experiments to minimise the number of animals.

(harms) to the animals.	
	This project will use Xenopus and mouse. Xenopus lays a few thousand eggs, which dramatically reduce the number of adult animals. Xenopus has less neurophysiological sensibility than mammals. Detailed molecular analysis will be performed using Xenopus. Mouse will be used for only specific objects, which have very strong evidence based on Xenopus and cell based in vitro work.

PROJECT 8

To study the epidemiology and pathogenesis of the diseases

KEYWORDS

Virus, salmonid, IHN, VHS, aquaculture

Summarise your project (1-2 sentences)

This project sets out to improve our understanding of two significant viruses of fish species used internationally in food production through aquaculture. This will, in turn, facilitate improved diagnostic tests, the development and refinement of vaccine products and, ultimately, improved animal welfare through the reduction of clinical and sub-clinical disease associated with these infectious agents.

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.

The overall aim of this project is to establish and apply experimental models of IHNV and VHSV infections to study the epidemiology and pathogenesis of the diseases. Specific objectives would be to:

1. Determine the infectious dose of virus by waterborne exposure
2. Determine the sites and relative contribution of different tissues and organ systems in shedding of virus into the aquatic environment.
3. Determine the potential for exposed fish to develop a carrier status and to transmit infection to in-contact fish.
4. To provide samples for development and comparative evaluation of diagnostic tests
5. To provide samples for studies on, and comparative evaluation of factors such as virus strains, fish strains, fish age, fish species, environment and husbandry on pathogenesis.

Outline the general project plan.

Bath, intraperitoneal and co-habitation challenge studies using different strains of IHN and VHS viruses. Staged culling of samples of the populations, from which tissues will harvested for subsequent laboratory analysis. Clinical observations will be analysed for the development of robust humane endpoints in future 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.

During the study, experimental animals will be infected with viruses through various routes. We expect clinical sins associated with infection to develop. These signs

include inappetance, reduced growth, colour change, increased respiratory effort, flashing and/or spiralling behaviour. Moribund fish will be primarily identified by loss of coordination, normal buoyancy and swimming ability and the development of these signs will be used as humane endpoints.

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

Improved understanding of the epidemiology and pathogenesis of IHN and VHS viruses. In turn, this will facilitate development of improved, rapid diagnostic tests and vaccine products which can be used in the control of these diseases. As a result, food production efficiency and the welfare of animals in aquaculture systems will be improved through the reduction of clinical and subclinical disease associated with these two infectious agents.

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.

Up to 6,000 salmonid fishes per year will be used. The advice of an experienced biostatistician will be sought to allow the minimum number of animals to be used in studies, whilst maintaining statistical significance and power in 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.

Virus stocks will all be grown up in tissue culture. However, in order that the epidemiology (study of spread of infection in populations of fish) and pathogenesis (study of disease development in fish) can be elucidated, it is essential that animals are used to determine the effect on the whole biological system.

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

While it is expected that clinical signs associated with infection will develop during cycles of infection, the information gathered in this study will help in the development of future end-points for similar studies. Technicians and scientists involved in this project have significant experience in viral infection models of fishes and will closely monitor animal welfare throughout the study to minimise suffering.

PROJECT 9	**Glucocerebrosidase Deficit in Parkinson's Disease**	
Key Words (max. 5 words)	Glucocerebrosidase, Gbal, Parkinson's disease, alpha-synuclein	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	Y	Translational and applied research
	Y	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	Y	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The mechanisms by which glucocerebrosiaase 1 *(GBAI)* mutations increase the risk for Parkinson's disease (PD) is unknown and is currently a major focus of research, as this offers not only insight into pathogenesis, but also the prospect of an entirely novel therapeutic strategy in PD. We propose to investigate the biochemical consequences of *Gbal* deficiency in brain tissue from the heterozygous *Gbal* knockout and heterozygous *GbaI(L444P)* mice. Further, *GBA1* mutations are associated with increased aipha-synuclein levels and impaired mitochondrial function, whilst aipha-synuclein over-expression and *PINKI* knockout reduced glucocerebrosidase (GCase) levels and activity. We hypothesise that *GBAI* mutations interact with and accelerate pathology related to alpha-synuclein and	

	mitochondrial dysfunction, and this forms the basis for how they increase risk for PD. We wish to test this hypothesis by creating novel mouse models that will simultaneously express the L444P mutation in the *Gbal* gene with either alpha-synuclein over-expression or the *Pinki* mutation. Studies to date indicate that alphasynuclein over-expressing and *Pinki* mutant mice each demonstrate only mild abnormalities of dopaminergic function, but do not express typical PD pathology, and so we predict that incorporation of the L444P mutation in the *Gbal* gene will enhance and accelerate the pathological and biochemical abnormalities. Finally, it has been recently shown that a single intrastriatal injection of alpha-synuclein fibrils into wildtype nontransgenic mice resulted in development of Parkinson's disease symptoms. To sum up, the overall objective of this project is to generate and investigate several mouse models that will reflect the biochemical interactions seen in PD patients and will prove to be useful for further molecular characterization of PD aetiology and potential therapies to modulate GCase activity.
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 completion of this study will provide a comprehensive and complimentary approach to discovering how GCase deficiency and mutation in the *GBAI* gene increase the risk for PD. It will also provide an answer whether different causal Parkinson's genes act in concert and together lead to more rapid development of Parkinson's disease symptoms. This study will result in generation of the new mouse models of PD, and subsequently will give the research community an opportunity to follow the development of Gbal-related pathological changes over time and in different areas of the brain and in peripheral tissue such as intestine. Also, these new mouse models will be used to test whether pharmacological chaperons that enhance GCase function (and are already used for a treatment of Gaucher disease) are also able to decrease and/or slow down alpha-synuclein pathology (so Parkinson's disease symptom). In future, these new models could

	be used for testing other potential therapies for PD patients, both those with and those without *GBAI* mutations, as well as for testing new drugs developed to correct *GBAI* mutations in Gaucher disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We wish to use mice in our studies, as they share many similarities with humans, both in terms of physiology and anatomy. We expect to use a few thousand mice over the entire duration of the 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?	Most of animals produced during the duration of the project are not likely to exhibit any harmful phenotype with the exception to some homozygous mice that are expected to die shortly after birth and mice that will be injected with aipha-synuclein fibrils that are predicted to develop pronounced alpha-synuclein pathology that will lead to much more rapid development Parkinson's disease symptoms. It is not envisaged that most of husbandry methods or procedures listed will cause any long term adverse effects. However, it is recognised that cutting the tail of a rodent or blood sampling is uncomfortable and this discomfort will be minimized by the use of anaesthesia at the point of collection. Also, mild local irritation and discomfort might be observed at the site of intraperitoneal, intravenous and subcutaneous injections. Haemostasis will be confirmed before the mice are returned to their home cages and monitored thereafter. Procedures that are conducted under terminal anaesthesia will ensure that the level of anaesthesia will be maintained at sufficient depth for the animal not to feel any pain. At the end of the project, mice will either be killed or kept alive at the designated establishment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim of this project is to determine whether mutations in the *Gbal* gene induce biochemical and pathological changes that would result in the development of Parkinson's disease in mice, so that these mice could be subsequently used for developing potential therapies to correct *Gbal* mutations (to modulate GCase activity). There is no feasible alternative that would replace the use of

	living animals that would allow achieving these objectives. This study has been designed to initially use cell culture models to test the influence of *Gbal* mutations on the lysosomal, mitochondrial and autophagy function. However, to determine the impact of *Gbal* mutations on a range of processes occurring simultaneously in such a complicated organ as brain, as well as to be able to test the potential adverse effects of the therapies developed to correct *Gbal* mutations on the whole organism, we need to test the effect of *Gbal* mutations in an in vivo model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the same outcome variable in all the in vivo studies. These are a continuous numerical data, in each study we will use genetically defined inbred stocks. In the analysis of our results we will use a 5% significance level and a statistical power of 95%. We will search an advice of a statistician on a number of animals that should be used to obtain statistically relevant results from behavioural tests.
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.	Choice of species and models: While there is currently no perfect mouse model of GBA 1-associated PD we have chosen heterozygous *Gbal* knockout and heterozygous *Gbal(L444P)* mice to study how the that GCase deficiency lead to biochemical abnormalities that are known to contribute to the pathogenesis of PD. The use of these two models will also allow us to study the mechanism behind the relationship between *GBAI* mutations and PD by testing both the hypothesis of *Gbal* loss-of-function (heterozygous *Gbal* knockout mice) and *Gbal* gain-of- function (heterozygous *Gbal(L444P)* mice). In order to investigate a relationship between alphasynuclein accumulation and *Gbal* deficiency as well as a relationship between *Pinkl-related* mitochondrial dysfunction and *Gbal* deficiency we aim to generate dual-transgenic animals. These animal studies will allow us to investigate biochemical interactions seen in Parkinson's disease (PD) patients and will prove to be useful for further

	molecular characterization of PD aetiology and potential therapies to modulate GCase activity. Where suitable lines of genetically altered animals already exist (heterozygous *Gbal* knockout, heterozygous *Gbal(L444P), Snca, Pinkl-/-* and *B6129SF1/J* mice) animals will be obtained from the relevant supplier. To generate dual-transgenic mice, we will cross animals from genetically modified lines (named above) obtained from the relevant supplier. It is unclear how many animals will be required to maintain an established line: however, we will measure production and breeding performance and ensure the minimum numbers of animals are used in the programme. The animals will be monitored for normal behaviour, posture and feeding and weighed on a regular basis. Any animal that shows any persistent behavioural abnormality or weight loss will be killed humanely.

PROJECT 10

Regulation of muscle stem cell function and metabolism

KEYWORDS

Adult stem cells; muscle; regeneration; ageing; metabolism

Summarise your project (1-2 sentences)

The aim of this project is to examine the mechanisms that control adult muscle stem cell function, and therefore subsequent muscle mass and contribution to whole body metabolism. We will use wild type and genetically modified mice and make multiple observations on muscle and its associated stem cells to understand the most important factors needed for stem cell fate 'decisions'.

• **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.**

Adult stem cells have the 'choice' to either self-renew or irreversibly form specialised cells (in this case muscle myofibres) and maintaining this balance is essential. Too much self-renewal can lead to cancer whereas insufficient self-renewal diminishes the stem cell population and ultimately impairs muscle regeneration in response to 'wear and tear' or acute injury, leading to reduced muscle mass.

The health of adult tissue is in large part dependent on the correct function of adult stem cells throughout life. In this regard, it is significant that cancer and sarcopenia (reduced muscle mass/strength) are diseases of age. Additionally, many muscle pathologies (such as muscular dystrophy) involved aberrant adult stem cell function.

Stem cell function is dictated by signals derived from within the cell (intrinsic) and outside the cell (extrinsic or environmental). We now know that, in the case of muscle satellite cells, environmental signalling is predominant in determining cell decisions, particularly during ageing (a 'young' environment can rejuvenate 'old' adult muscle stem cells). Thus, a key aim of this project is to determine what contributes to this external signalling, and how is it translated into changed cell gene expression and hence cell fate choice (self-renew/differentiate, survive/die, remain in the stem cell 'home' - called a niche/migrate.

A key function of muscle is in glucose metabolism; one of the first signs of type 2 diabetes is impaired muscle glucose utilisation. Thus it is relevant to assess whole body metabolism when muscle mass or metabolic rate have changed.

• **Outline the general project plan.**

Initial studies are performed using muscle stem cell lines, to provide preliminary

evidence that a candidate mechanism/pathway/extracellular signal does indeed regulate satellite cell and hence muscle function. We also confirm these findings using ‘proper’ satellite cells isolated from wild type mice.

When satisfied that our candidate may have a key role in satellite cell function, we then proceed to establish its function *in vivo.* This is the only meaningful way to assess stem cell behaviour properly. Mice are generated to either have none of our candidate in muscle (termed a knockout mouse), or have a changed form of the candidate (knockin) or have extra levels of the candidate (overexpressing). We have sophisticated methods to ensure that we can control where (and even when) we manipulate our candidate.

Having generated these novel genetically modified mouse strains, we then assess the ability of muscle to grow and develop, the number and behaviour of muscle satellite cells, the ability of muscle to regenerate following a modest (and reversible) injury, and (if appropriate) consequences to muscle, and therefore whole animal, glucose metabolism.

Since we are interested in muscle function throughout life, some of these studies will be performed in ‘old’ mice (18-24 months), or in response to changed nutrition/hormonal status.

• **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.** Most of the procedures employed in this project do not cause more than transient discomfort to the animals or lasting harm.

Usually the genetically modified mice we generate exhibit only mild changes in physiology. However, it is possible that muscle growth (and therefore whole body) growth may be more severely compromised, for example by up to 50%. This results in weaker animals, which need additional care and are culled if they show signs of discomfort.

A very important aspect of this project is the assessment of the ability and efficiency of muscle regeneration (muscre must regenerate throughout life to maintain tissue mass and this ability decreases greatly with age). Regeneration is modelled by inducing a modest (and reversible) muscle injury, using well-established protocols. This is either snake venom injection (breaks down muscle fibres but does not affect satellite cells which regrow the muscle) or a freeze for a few seconds (kills all myofibres and cells, and so tests the ability of satellite cells to travel from

neighbouring fibres and repair).

• **Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.**
This project will advance our understanding of muscle stem cell (and therefore other tissue stem cell) function in a variety of physiological conditions. Muscle frailty is a major problem in the elderly, leading to falls, lack of confidence, isolation and possibly depression. The research within this project should lead to ideas for the development of strategies to reduce diminished muscle function during old age.

• **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.**
We estimate we will use up to 3,000 mice per year during this study. Mice are used because they provide a good model for many aspects of human muscle function, they provide sufficient material for analysis, and because advanced genetic models already exist (thus avoiding needless repetition). One reason that we use this number of mice is that some complex breeding crosses only generate a small number of mice carrying a revealing gene combination. However we do try to ensure as much efficiency as possible (e.g. production of 'control' and 'test' animals within each litter).

• **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.**
Replacement: satellite cells do not respond in culture in the same way that they do *in vivo,* meaning that incorrect conclusions would be obtained from only culture work. In addition, the complex 3D structure of muscle can only be investigated properly within the context of the animal. *Reduction:* we make multiple observations on each animal, using several muscles, so that output is maximised. Pilot studies are always performed initially, and the number of animals used is based on knowledge of the error of the determination. Where appropriate, statistical advice is sought. Should a representative cell culture model become available, we would use it. *Refinement:* as discussed in the previous section, mice are the most appropriate model animal.

• **Explain why the protocols and the way they are carried out should involve the least suffering.**
All protocols are considered carefully for the expected benefit versus the cost to the animal. We follow best practice in all situations, ensuring appropriate anaesthetic and analgesic, when required.

PROJECT 11	**Induction and resolution of renal inflammation**	
Key Words (max. 5 words)	Kidney, inflammation, autoimmunity, vasculitis, leukocytes	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	ANCA-associated systemic vasculitis (AASV) affects over 1000 people/year in the UK, approximately half of whom will die or develop permanent kidney failure as a direct result of the disease or its treatment. A major breakthrough in our understanding of the mechanism of kidney injury in this disease was the discovery that a specific form of antibody (termed ANCA) is usually present in AASV, and the appreciation that this antibody is likely to be responsible for initiating damage to blood vessels in the kidney. However, we have only a minor understanding how inflammation can resolve and how we may harness the natural methods that regulate inflammation in healthy individuals. Our work to date has found that certain proteins made by particular white blood cells can either lead to promotion or resolution of inflammation. We wish to investigate if we can translate some of these findings to develop new therapeutic targets for clinical application, as well as understanding fundamental aspects of how	

	inflammation is naturally controlled.
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)?	This programme of work will extend our knowledge of the critical biological events that cause kidney failure in patients with AASV, and other forms of renal inflammation and will potentially translate our findings to develop novel therapeutic targets that could be applied to patients with various forms of kidney disease or those at high risk of developing kidney disease (such as patients undergoing certain surgical procedures).
What species and approximate numbers of animals do you expect to use over what period of time?	The studies will be carried out in mice and rats. The primary determinant of this choice was driven by the fact that we are building upon previous work that developed these models of kidney inflammation in these species. These species are sufficiently close to human physiology, but yet sufficiently far down the sentient scale, to allow relevant conclusions to be drawn regarding what may be happening in patients with varied forms of renal inflammation, while still avoiding use of higher animals such as non-human primates. The explosion in the availability of genetically modified mice now allows us to dissect in detail disease mechanisms that were hitherto impossible. We expect to use a total of just under 2900 animals over the 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?	The development of renal failure is generally without symptoms. The models we use do not induce severe renal failure but significant renal inflammation. The induction of various forms of renal inflammation requires administration of substances by injection, but these are performed by experienced operators and suffering is kept to a minimum. At the end of the protocol animals are humanely sacrificed and the extent of their kidney inflammation assessed by histology, biochemistry and immune monitoring.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are at a critical juncture in the study of AASV and other forms of renal inflammation, A detailed hypothetical schema describing the potential events that may occur in the kidney resulting in injury to it has been developed based on 20 years of experiments involving cells in test- tubes. As the last

	few years has witnessed the development of novel animal models of AASV, and of other forms of renal inflammation, we are now in a position to begin exploring the disease pathways in living organisms. The events that lead to blood vessel injury in the kidney in AASV and following ischemic or drug induced inflammation are certainly complex, involving numerous cell types, antibodies and immune system proteins, the integration of which would be impossible to achieve by replacement with test-tube studies. Additionally, novel therapies deriving from new insights gained by studying these animal models will need to be tested in such models before being considered for use in human patients with AASV.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Through thoughtful experimental design and careful articulation of the specific scientific questions to be answered, we will reduce the amount of animals used in this programme to the smallest possible. Examples of ways that animal use can be minimised include combining 2 or more questions into one experiment, careful storage and cataloguing of all biological samples to facilitate future studies without having to repeat experiments, and accurate statistical consideration of the numbers of animals in each experiment so that the experiment has a realistic chance of answering the question being posed. The studies have been refined so that they are of minimal severity wherever possible, that anaesthetic protocols are reliable and safe, that immunisation routes cause the least amount of discomfort and that animal husbandry is of a sufficient standard to maintain the animals in an environment as close as possible to their natural environment. For example, we have established that the quality of the model is not improved by repeated booster immunisations, so these are now omitted.
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	The studies will be carried out in mice and rats. The primary determinant of this choice was driven by the fact that we are building upon previous work that developed these models in these particular species. These species are sufficiently close to human physiology, but yet sufficiently far down the sentient scale, to allow relevant conclusions to be drawn

measures you will take to minimise welfare costs (harms) to the animals.	regarding what may be happening in patients with AASV or other forms of renal inflammation under different circumstances, while still avoiding use of higher animals such as non-human primates. The explosion in the availability of genetically modified mice now allows us to dissect in detail disease mechanisms that were hitherto impossible.

PROJECT 12	**Regulation of T cell immunity and autoimmunity**	
Key Words (max. 5 words)	diabetes, autoimmunity, T-cells, immune regulation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our immune system is fundamental for protection from infectious disease; without it we would quickly succumb to infection. An important feature of the immune system is its ability to discriminate between foreign threats such as bacteria and viruses and our host tissues. Type one diabetes occurs when this process breaks down and the immune system attacks the Insulin producing islet cells in the pancreas. Insulin production controls the uptake of sugar from the blood and so must be replaced by multiple daily injections in order for the body to use sugars consumed in our diet. Excess sugar in the blood leads to serious long term complications such as nerve and small blood vessel damage. We are making great progress in our understanding of the ways in which the immune system manages its responses but there remains much which is still unclear. In order to dissect the diabetes associated immune	

	response, we have developed a mouse model in which we have altered the immune system so that it attacks the pancreas. These mice develop diabetes in a manner that is similar to diabetes in humans. For example, the same types of immune cells appear to enter the pancreas in these mice and in diabetes patients. In addition, antibodies specific for pancreas proteins can be found in the blood of both the mice and humans during diabetes development. Because we have an animal model to study diabetes onset, we can manipulate it to ask which genetic pathways are important for causing or preventing disease.
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)?	We hope that by the identification and careful study of the cells and process which lead to the onset or prevention of diabetes in the mouse model we will be able to accurately predict the processes involved in human diabetes onset. We work closely with world-class clinicians allowing us to quickly validate these hypotheses using human patient samples. It is our aim to ultimately develop ways in which to manipulate these key control pathways so as to prevent the onset of autoimmunity.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice in this study as they provide a very good model of the human immune response. We expect to use approximately 14,000 mice during the entire study period (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?	The vast majority of the mice used during this study will not be subject to any adverse conditions. The exceptions to this include some strains of mice which will develop spontaneous autoimmunity and some mice which develop autoimmunity as a result of cell transfer. Our assessment is that these mice will experience moderate discomfort. At the end of the project, mice will either be killed or kept alive at the designated establishment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	This project aims to identify novel cells and mechanisms for the modulation of the anti-islet autoimmune response. In order do this it is imperative that we are able to access the cells at the point at which the immune system makes the crucial decision

use non-animal alternatives	of whether or not to initiate a response. For a pancreatic disease, such as Type one diabetes, this is in the pancreatic draining lymph node. This cannot be accessed in human patients. In order to test the involvement of particular pathways of interest it is also essential to be able to block or genetically manipulate them.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise the use of animals in experiments that do not turn out to be informative, pilot experiments are performed. This ensures that large group sizes are not used in experiments that are unlikely to yield useful information. Animal numbers will be minimised by keeping records of the number of cells typically obtained from donor mice bearing different genotypes. This allows accurate planning of the number of donor mice of each type required in order to provide cells for a given number of recipients.
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.	To study T cell responses to self proteins it is necessary to target the immune response against a self tissue in a manner that has the potential to cause autoimmune disease. By choosing to target the pancreatic islets, we have ensured that we can test the degree of pancreas destruction without killing the animal. This is because as the insulin-producing pancreatic islets become destroyed, the animal loses its ability to control its blood glucose levels. By monitoring blood glucose levels (using the same glucometer used by type 1 diabetes patients) we can therefore obtain accurate kinetic data from a single animal showing pancreas destruction over time. Such an accurate measure of disease progression enables close monitoring of disease progression and thus prevents unnecessary suffering to the animal.

PROJECT 13	**Control of excitable cell (nerve/muscle) function**	
Key Words (max. 5 words)	control of excitable cell function	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to characterise the fundamental biochemical and electrical pathways in cardiac and smooth muscle that are responsible for their normal contractile and electrical activity, to understand how nerve transmitters control the function of these muscles, and how other tissues closely associated with these muscles affect their function. Many of these fundamental aspects of muscle function remain unknown and this project aims to clarify them and the influence of external modulators.	
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)?	These fundamental data will then be used to compare equivalent data from tissue with pathological function to elucidate the fundamental basis of organ dysfunction that in humans are often fatal and at other tmes lead to a severe reduction in quality of life. In particular the work will concentrate on the heart and the lower urinary tract (bladder and outflow) to provide the basis for understanding cardiac arrhythmias and failure, as well as overactive bladder syndrome.	

What species and approximate numbers of animals do you expect to use over what period of time?	Guinea-pigs 400 Rats 100 Mice 100
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?	All procedures are classified as MILD The only adverse effects may be slight discomfiture during intraperitoneal injection of heparin. The agent itself causes no adverse effects. Animals will be humanely killed at the end of the procedures
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim of the project is to characterise the physiological properties of multicellular preparations and cells isolated from these preparations. Because tissues are complex, consisting of many cell types (nerves, muscle cells, epithelial cells, fibroblasts, interstitial cells, etc) and structured in a defined topological arrangement, It is important to work with intact multicellular preparations to characterise their particular functional properties. This work is combined with equivalent studies using human tissue. However, human tissue comes from a variety of pathological states that makes characterisation of fundamental processes difficult. The animal experiments are therefore necessary to provide a scientific basis for tissue function so that targeted experiments can be done on human tissue. Combined, the use of animal arid human tissue will enormously speed up the identification of therapeutic targets to control pathologies associated with these organ dysfurictions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Each experiment is performed a sufficient number of times to ensure that the result is statistically meaningful but not too many times when additional information would provide no further statistical benefit. Numbers of repeats are based on a power calculation that estimate the optimum number using the above criteria. A very important aspect of this project is that the same animal may be used as a source of tissue for two branches of investigation carried out in my laboratory, namely experiments on the heart and on the lower urinary tract. This reduces greatly the total

	number of animals required, as there is collaboration between the two groups to co-ordinate animal use.
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 animals to be used (guinea-pigs, rats and mice) share many similarities with human tissue and so the extrapolation of these findings to human conditions will be effective and efficient. All animals will be housed in a safe experimental biology unit as the centre where experiments will be performed. Animals are monitored daily for their general health by skilled technicians and any animals showing sickness that persists over several hours will be humanely killed. The procedure to be employed in this project is MILD and no serious adverse effects are anticipated.

PROJECT 14	**Neurobiology of sensory processing**	
Key Words (max. 5 words)	Sensory processing, feedback, attention	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) (Part 1)	An easy way to imagine how we perceive the visual world is that the eyes project an image of the world on the cortex of the brain and that we “see” this image. Like all things in life it is not this simple at all. In fact, what we see is a selected portion of the visual spectrum sampled by the receptors in our eyes and a reconstruction of the input that comes from that, assembled in terms of our visual experience in ways that do not always reflect what is there, rather what we think and expect is there. Our perception of what we “see can thus be influenced by prior knowledge and expectation and many common visual illusions fool our brain into perceiving non-existent contours or objects. Similarly, many patients are unaware of deficits in their vision resulting from retinal damage as their brain attempts to “fill-in’ these holes on the basis of the surrounding information in the visual scene. Essentially, we see what we think should be there. What is going on? Input from the eyes is relayed via a structure called the visual thalamus to the primary	

	visual cortex, but the visual cortex sends feedback connections back down to the visual thalamus. Numerically these exceed those arising from the eyes. Similarly the primary visual cortex relays visual input to other cortical areas that specialize in processing different image aspects such as colour or motion and each of these higher areas projects back to earlierievels. It seems that in the first instance each point in the visual image is processed by sets of local feature detectors that pick out a range of local image attributes such as orientation, direction of motion, colour and depth. The outputs of all these local detectors are then further processed and put together in different combinations through channels that specialise in certain aspects of the image such as the motion of its parts or the links between colour and form. The thousand dollar question is how is all this brought together into our unified percept of the world outside our head? In part the answer lies in the fact that the highest cortical visual centres integrate these many views but it involves other things too. One factor is that feedback pathways return the views of the world assembled at higher levels to lower levels and thus change the way the earlier levels assemble the information. This serves to integrate the information going back up the visual pathway.
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) (Part 2)	The overarching hypothesis driving this proposal is that top down connections from the visual cortex to the visual thalamus modify what is relayed to the cortical mechanism to reflect the attentional focus and priors of higher cortical levels. In so doing we suggest they integrate the thalamus into the ongoing representation of the visual world. Testing this hypothesis, for which we have good preliminary evidence, necessitates a reappraisal of the response characteristics of cells in the visual thalamus based on their responses to classes of visual stimuli and behavioural task normally used to explore higher-level visual function.
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)?	This project has many implications for both how we understand the basic neurobiology of vision and sensory processing and how we treat patients with problems such as lesions to the visual pathway or retina and those experiencing hallucinations after retinal damage or more complex diseases such as

	schizophrenia. This work will contribute in an original way to our understanding of the processes of vision by probing a range of higher order visual processes standing in the interface between cortex and thalamus that have not been previously recognized. The circuitry that sits at the interface between thalamus and cortex is broadly common to all mammalian sensory systems and the recognition of what happens inthe visual system should generalize across systems. An evaluation of the way higher brain mechanisms modify and overwrite information transmitted from the thalamic locus providing the brain's window on the world will add to our appreciation of the way the brain models the world and underline the importance of learnt priors in shaping our perception. The work will advance our knowledge of a key brain mechanism with relevance to health and disease. By providing further insight into how the visual system fills in" and compensates for the loss of input from the damaged regions, it will help define ways of recognizing and minimizing the perceptual deficit. It will help define the limits of what can be achieved and the ways in which "filling in" across retinal scotomas can lead to errors in judgment. It also identifies a way in which cortical feedback might generate hallucinatory input from the denervated thalamus in patients with AMD. Feedback systems are thought to functional abnormally in schizophrenia and this research identifies a route whereby the cortical mechanism may generate hallucinations. By adding to the knowledge of how the brain musters finite resources to tackle very complex problems it will guide strategies driving computational approaches to vision and may suggests ways of optimising system performance with finite resources in ways applicable to those developing artificial visual systems for computational industrial and medical systems.
What species and approximate numbers of animals do you expect to use over what period of time?	Primates (macaques), 9 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse	Good husbandry and care practices based on best practice in the field and Veterinary advice will be used throughout. Animals will be observed sufficiently often

effects and the likely/expected level of severity? What will happen to the animals at the end?	for any adverse effects to be detected at an early stage and steps taken to minimise them. Animals will be humanely killed at the end of the experiments as it will be necessary to correlate the recorded data with a histological reconstruction of recording sites.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no possible substitute for animal use as evaluating the interplay between feedforward and feedback circuits, behavioural relevance and attentional modulation can only be addressed in the intact animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the minimum number of animals to support the objectives. Experiments are carefully planned and phased, and organisation of staff, equipment and techniques are optimised. Wherever possible, recordings are taken from multiple cells in order to maximise the amount and quality of the data.
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.	These experiments will be performed in primates as the visual system of primates (including man) is substantially different to that of other mammals. Experiments are designed to involve the least invasive and lowest number of interventions commensurate with the objectives. Protocols are continuously refined to minimise their severity. Animals are group housed in cages with appropriate environmental enrichment and will not be single housed unless there is a clear veterinary or welfare need.

PROJECT 15

Investigation of the Role of Nox and Reactive Oxygen Species in the Pathogenesis of Metabolic Disorders

KEYWORDS

Cardiovascular diseases; oxidative stress; type 2 diabetes; NADPH oxidase

The overall aim of this project is to discover the roles of Nox and its product, reactive oxygen species (ROS) in the development of metabolic disorders and cardiovascular diseases for the purpose to discover the new targets to treat or to prevent these diseases.

Cardiovascular disease is a major cause of death and illness in subjects with obesity, insulin resistance and type II diabetes and represents a major healthcare problem. Studies from us and others have discovered that endothelial dysfunction characterised by excessive production of reactive oxygen species (oxidative stress) by an enzyme called NADPH oxidase (Nox) is a feature in the early stage of the development of cardiovascular diseases. Compelling evidence has shown that factors such as angiotensin II, high blood glucose and high fat (or cholesterol) diet may activate Nox and cause systemic oxidative stress, endothelial dysfunction and contribute to the development of insulin-resistance, obesity, type-II diabetes, hypertension and atherosclerosis. However, the role and the mechanism(s) of Nox activation in these diseased conditions are largely unknown.

Cardiovascular diseases and metabolic disorders are complicated pathophysiological processes that involve integrated malfunctions of multiple organs/tissues and cells (paracrine and endocrine) in a living animal or in human. We have to use animal models of these diseases to understand the role and the mechanisms of Nox and ROS in the pathogenesis of hypertension, atherosclerosis, obesity, insulin-resistance, type 2 diabetes and other cardiovascular complications. There is no alterative way to achieve this. Every alternative way (such as in vitro cell culture and ex vivo organ functional assessment) has been considered and applied in our project whenever it is possible.

During our study mice with genetic modifications of Nox deficiency or cardiovascular disease related gene will be bred. These mice will then either be used to provide tissues for our work. Or they will be used in studies for Angil-induced hypertension or high-fat diet induced obesity, insulin resistance and type 2 diabetes. The numbers and sizes of the groups of mice will be determined by statistical analysis, but will be kept to the minimum required for significant results. We will try to use the same animal for several set of experiments such as for cardiac stem cell isolation (heart), for vessel contraction (aorta), for bone marrow cell isolation (legs) to reduce the number

of animals.

Our research is to reduce oxidative stress which is expected to protect animals from endothelial dysfunction and reduce diseased symptoms. This project will provide insight into the role and the mechanisms of Nox2 and its product, reactive oxygen species, in the pathogenesis of metabolic diseases and cardiovascular diseases. The crucial information from this project will be used to discover the new targets for the development of novel therapies for patients.

PROJECT 16	**Lung repair and regeneration**	
Key Words (max. 5 words)	Injury, repair, regeneration, cell fate.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)		Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this license are to identify and develop novel therapies aimed at modulating/promoting repair and regeneration of functional tissue in a variety of respiratory diseases.	
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 benefits likely to derive from this project are a greater understanding of the molecular mechanisms involved in lung repair and regeneration in response to injury and therapies that could improve the prognosis for patients suffering from chronic lung diseases which have damage and destruction of lung tissue as a component.	

What species and approximate numbers of animals do you expect to use over what period of time?	Mice Up to 5000 over five years plus up to 800 mice in short term pilot studies.
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?	Animals undergoing sulphur dioxide inhalation are expected to experience discomfort during exposure and will become inactive and hunched. Following exposure animals are expected to display the following clinical signs for 48 — 72 hours: a. Altered respiration rate with intermittent “chattering”. b. Piloerection. c. Lethargy, with little peer interaction. d. Weight loss of up to 20% of body weight, measured against age-matched controls. (Average weight loss is 10%). e. Eyes kept partially closed. Following this period there will be a rapid return to normal behaviour. This is a severe protocol. Animals undergoing detergent installation are reported to lose on average 10% of body weight over the first 48 hours following exposure with a more severe weight loss (up to 20%) observed for 1% of animals. Animals are somewhat lethargic and intermittently change their respiration pattern (hyperventilating). After this period restoration of normal behaviour and weight is rapid. This is a severe protocol. Animals exposed to influenza virus are expected, for the initial 7-10 days post-infection, to show signs of distress including weight loss, pilorection and changes in respiratory rate, after which the animals should recover quickly. Where recovery does not take place in the normal fashion, animals will have to be humanely culled to prevent further morbidity. This is presently a severe procedure, pending the outcome of pilot studies being conducted under a different project licence. At the end of all protocols animals will be culled and tissues removed for analysis.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The program of cell proliferation and differentiation which allows the lung to repair and regenerate following injury is highly complex and involves a multitude of interactions between different cell types including epithelial, endothelial and mesenchymal cells.It is not currently feasible to adequately model the complexity of these responses using in vitro systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A novel 3D organotypic primary human airway culture system, that has been developed 'in-house', will be used for both target identification and drug screening/optimization. This means that studies in vivo will only be considered for drugs/targets that have demonstrated the desired behaviours in vitro. The animal numbers chosen for each study will reflect the variability of the modeL, so that each experiment is designed to ensure that meaningful data can be obtained. Where necessary, power analysis will be performed to ensure that groups are of the minimal size required to produce statistically meaningful data. Statisticians will be consulted as necessary. Wherever possible and appropriate, multiple endpoints will be measured from each animal to ensure that experiments will not have to be repeated to assess different parameters.
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. (Part 1)	For this project mice will predominantly be used for a number of reasons. The mouse trachea closely resembles the majority of airway generations of the human lung. In addition, the composition of alveolus in mice is thought to be very similar to that of humans. This means that the study of stem cell biology and cell fate in the mouse trachea and alveolus is relevant to both human airways and gas-exchange tissues respectively. In addition, the use of mice will allow us to benefit from the availability of GM strains which will be invaluable for the validation of mechanisms and targets we identify from in vitro studies as being involved in cell fate decisions following airway epithelial injury and the overwhelming majority of published work on the models utilised in this project has been performed in murine systems. The sulphur dioxide inhalation and detergent instillation

	models are well-defined models of tracheobronchial injury/repair, leading to a rapid induction of basal cell proliferation and the eventual restoration of a histologically normal epithelium. In comparison with the alternatives. (e.g. acid aspiraUon, chlorine gas inhalation) both models have lesser welfare implications for the animals causing behavioural changes and weight loss for limited periods post-exposure. Both models will be run initially as pilots during which refinements will be introduced (e.g. reduced exposure, liquid diets, heat pads etc) to determine which is most suitable in terms of the scientific results produced with the fewest welfare implications. This will be selected as the methodology of choice for future studies. The basal cell is the key epithelial progenitor cell in the conducting airway and it's proliferation and cell fate is an important focus of this project. These models will thus enable the project's objectives to be achieved with the minimum of animal welfare implications.
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. (Part 2)	Lung exposure of mice to influenza virus induces wide-spread destruction of airway epithelial cells, including in the alveoli, by 7 days. Unlike other models of alveolar injury, this is followed by a largely complete restoration of normal lung histology through airway repair and regeneration. This makes it the most appropriate model for the investigation of repair and regeneration in the alveoli following injury. Post exposure monitoring will be increased over the standard monitoring regime and animals will be monitored closely for signs of ill health. A scoring sheet system will be put in place to record weight loss and other signs of distress, for example piloerection, hunching and altered respiratory rate and used in combination with periodic measurements of tissue oxygen saturation in order to closely monitor the animal welfare daily and ensure welfare costs are minimised.

PROJECT 17

Skeletal phenotypes in health and disease

KEYWORDS

Skeleton, osteoclasts, osteoblasts, honnonal, stress

Our skeleton is continually repaired and adapts to the forces placed upon it. The skeleton has a built-in “repair kit” that consists of a team of mechanics (the bone makers, or osteoblasts) and some large scouts (bone eaters, or osteoclasts) that seek out the damage. These two teams need to work together to make sure that the total amount of bone in the skeleton is right and that all wear and tear is repaired perfectly. When one team works harder than the other the balance between bone formation and bone removal is disturbed. We wish to better understand such conditions which are often caused by specific genetic mutations.

The two diseases we are interested in are osteopetrosis, in which bone removal is defective and Paget’ s disease of bone, in which both osteoclasts and osteoblasts work too hard and bone is not as strong as it should be. Our studies are largely done using bone cells cultured from patients or volunteers or using human cell lines in which we have expressed the same mutated gene, or altered the expression of the gene. These investigations are complemented by studies in mice and rats.

Mice are suitable for our studies, because mouse lines have already been generated in which the genes of interest are altered and because mice (and rats) are good models in which to study bone physiology. In total we will use 4000 mice. We also use 70 rats, as one of the genes under study is naturally altered in an existing rat strain we use as a model for one type of human osteopetrosis. We examine bone tissue from the animals at specific ages using imaging methods and we isolate bone cells for culture and functional tests after euthanizing the animal. Occasionally we need to perform surgery to induce a honnonal stress on the animals that will lead to activation of the osteoclasts. This will demonstrate whether the osteoclasts respond normally in the presence of the mutated gene. We will do this in 80 mice at most.

Version 1.3 In addition, we study bone cells to get better knowledge of the basic principles by which they function. We are particularly interested in the ways in which the bone cells move substances through the cells and from inside the cell to outside. We can do much of this work with osteoclasts cultured from blood of human volunteers, or isolated from human bone removed during surgery, for example after trauma or during joint replacement. A problem is, however, that the bone obtained from patient undergoing surgery is often diseased. We therefore in parallel use bone cells obtained from rodents and young rabbits (100 rabbit pups maximally).

Our studies will provide new knowledge on the way in which bone cells function and how mutations seen in osteopetrosis and Paget's disease act to disrupt normal bone cell function. These results will benefit patients, while new knowledge about skeletal cells benefits humans and animals.

PROJECT 18

Epithelial regeneration and carcinogenesis

KEYWORDS

Lung, thymus, regeneration, cancer, repair

This project will use animal models to establish mechanisms of thymus and lung homeostasis, regeneration, and tumorigenesis that will have clinical relevance for improving human lung and thymus regeneration and cancer treatment.

To date, we have made significant contributions to epithelial organ homeostasis, repair, regeneration, and disease research. However, we still do not know many of the cellular and molecular causes of epithelial organ disease or the ways in which individual genes influence these disorders. The use of animal models is necessary to determine these processes.

Prior to any animal experiment we will first perform in vitro studies to address these research questions. These studies will involve cellular growth and differentiation assays, in vitro organoid formation assays, and cellular death and apoptosis models that will help us minimize the number of experimental procedures in animals we must perform. Each proposed animal experiment will then undergo a rigorous assessment of potential scientific and clinical benefits, expected and potential adverse outcomes, and statistical validity.

Experiments will answer one of the following questions:
1) Establish whether and how changes to gene expression in the lung and thymus influence organ homeostasis.
2) Determine whether and how the manipulation of specific genes influences lung and thymus cancer formation
3) Address whether and how specific cells, genes, and signalling pathways influence lung and thymus tumour growth, metastatic potential, cellular regenerative capacity, and long-term homeostasis.
4) Establish whether and how tissue damage influences lung and thymus homeostasis, repair and regeneration.

These procedures should not result in more than moderate pain, suffering or distress to the animals. Some examples of agents to be used to answer these questions include bleornycin, naphthalene, polidocanol, benzo-a-pyrene, doxycycline, DMBAJTPA, and NTCU. Appropriate pain relief will be used to minimise the effects of surgical procedures and the effects of the genetic alterations for the majority of animals will be negligible and of mild severity. These effects and the numbers of animals used will be minimised by appropriate breeding, husbandry and/or veterinary measures.

The mouse provides an appropriate species for these experiments as a wide variety of genetically modified mouse models are available to study epithelial organ disease

and its basic biological and pathological processes are similar to those in humans.

Overall, this work will improve our understanding of which cells are responsible for intrinsic human lung and thymus homeostasis as well as assist in developing novel organ regeneration strategies. These will help shape friture surgical strategies that are needed for individuals with irreversible tissue injury. Second, our research will help define the specific cells and signalling pathways associated with development of human lung and thymus cancers. This will aid in the development of novel human clinical cancer therapies. Finally, these studies will help develop new targets for future clinical cancer management.

PROJECT 19	**Transcription factors in development And disease**	
Key Words (max. 5 words)	Transcription factor, gene expression, development, disease, normal cell death	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	Y	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand how interactions between key regulators control normal development and functions and changes that lead to disease applicable to humans. The complexity in higher organisms such as mammals means that there has to be tight regulation of cellular processes that control cell division, specialisation and death. All these processes are controlled by genes, which encode for proteins that are produced in different cells. Such genes, in turn, are highly regulated by transcription factors (TF5), which are proteins that can act as master regulators to switch genes, on or off. Such TFs control multiple genes and as such changes in TFs can alter cell fate and lead to diseases. These studies aim to: *(1)* analyse how different families of TFs control survival and death in specific cells (2) analyse how their	

	effects are changed by interaction with other proteins (3) identify changes that are linked with disease (heart disease, obesity, cancers). Such results are crucial for understanding what controls normal development and function, identify changes that cause diseases and determine if such regulators be used for therapeutic applications to prevent or treat diseases in humans.
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)?	Results of *in-vitro* studies have shown that key regulators control survival, growth and behaviour in specific cells in normal development and health by regulating specific genes. When disrupted, this process can cause diseases that are relevant to human health. Because of the tremendous complexity of mammalian systems, it is essential that such key regulators are analysed in an animal model that will more closely mimic the cell-cell interactions and signalling that occurs in the whole organism. Therefore, this study will use of in-vivo animal (mouse or rat) models that have been well validated as model systems for analysing specific aspects of normal development and/or diseases. Furthermore, by manipulating such factors in these animal models, using standard techniques, we can test how this different regulators control gene expression and cell fate in diverse tissues and determine how such effects can be modified by interactions with other cellular factors.
What species and approximate numbers of animals do you expect to use over what period of time?	Numbers will vary depending on the experimental studies to be undertaken at specific times, but in general use will be approximately 1000 mice/year. Before undertaking any studies, experiments would be carefully planned (with the help of statistician as necessary), in order to assess numbers /control groups of mice that will be required to give rise to statistically significant results. With careful planning and execution of these experiments we should be able to keep the numbers of animals used for experimental procedures to a minimum.
In the context of what you propose to do to the animals,	Most of the procedures for maintenance of animals in this project involve standard animal husbandry.

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	However, additional procedures will be required to test specific hypotheses that are proposed and these will include administration of anaesthetic for surgery or interventions (dietary or drug). The adverse side effects may include pain/discomfort, local infection/inflammation. Since proposed the studies are based on well established protocols and procedures, these should pose
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal models will only be used when there are no alternative in vitro systems that can be utilised. In many cases, positive results from cell lines have been validated in primary cell culture (e.g. cardiac cells, sensory neurons) prior to animal studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Such serial assessment and paired analysis improves statistical power and also helps to reduce animal numbers required to optimise time points, dosage, duration of treatment etc. Any results from animal models will be confirmed in appropriate cell lines, where possible in order to refine and reduce the numbers of animals to be used in these studies.
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.	In order to minimise adverse effect, trained staff will be involved in undertaking procedures, and in closely observing all experimental animals. Immediate action will be undertaken to relieve any suffering and animals will be culled if necessary.

PROJECT 20	**Development of healthy oocytes and embryos in mammals**	
Key Words (max. 5 words)	Fertility, eggs, embryos, chromosome-segregation, early development	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	N	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	It is well established that an inability to generate healthy fertilisable eggs is a major cause of human infertility. This is particularly the case as women get older. The effect of maternal age upon the quality of the egg is clearly seen in in vitro fertilisation (IVF) clinics, where patients who undergo an IVF cycle using eggs from younger donor mothers are far more likely to conceive than women of similar age using their own eggs. Although it is well known that the health of the egg is a major cause of infertility, we understand very little about how eggs are normally generated. The aim of this project is to examine the mechanisms of egg development, particularly focussing on three aspects that we hypothesise are key to fertility. For each of these three aspects we will perform investigations in eggs from young mice to determine how these mechanisms work, and perform some investigations in eggs from older mice to understand how these mechanisms change with	

	increasing maternal age. These three aspects are: 1.Oocyte **chromosomal status.** It is known that eggs from older mothers have a high likelihood of having the wrong number of chromosomes, and that these eggs are highly unlikely to lead to a healthy baby. We will investigate the mechanisms by which chromosome number is normally controlled in oocytes, and investigate a number of candidate reasons as to why this might go wrong, especially in oocytes from older mothers. 2. Mitochondrial function. Mitochondria are the powerhouse' of the cell, providing the energy required for fuelling the activities of the cell (termed ATP). It is logical that a defect in producing energy would lead to a less healthy cell, and there are several lines of evidence that mitochondrial dysfunction is associated with unhealthy eggs. We will investigate the role of mitochondria in the development of the egg, including how they are organised, and how they generate energy during oocyte development. The DNA-Damage-Response. The genetic information within a cell is packaged in chromosomes as DNA. Maintaining the integrity of this DNA is essential for the life of an organism, or the health of an egg. In the case that DNA becomes damaged, most cells have a surveillance system called the DNA-Damage-Response (DDR) that detects and deals with the damage. How this is achieved in eggs is poorly understood, and we have preliminary data that suggests that eggs from older mothers are incapable of responding to damage. We will investigate the means by which eggs deal with DNA damage, and determine why this is apparently deficient in eggs from older mothers.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Our lab aims to understand the biology of the egg and early embryo, so as to shed light on the reasons why some women, particularly those of advancing age, struggle to conceive. The age at which women have their first child has rapidly increased over the last — 30 years, and so understanding how healthy eggs are

project)?	generated, and the reasons why this process goes wrong is increasingly going to impact health and wealth in the coming decades. In the long term, our work is positioned to help understand the basis and possible treatment avenues for female infertility.
What species and approximate numbers of animals do you expect to use over what period of time?	—2200 mice/year
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?	Two procedures will be employed. The first is to administer hormones to mice. This is performed to increase the numbers of oocytes and embryos available for a given experiment, and thereby reduce the number of animals required. The hormones are delivered by intra-peritorieal injection in a small volume of saline solution. This causes no suffering or distress to the animals. Mice are subsequently sacrificed by cervical dislocation, the most humane available method, in order to harvest the oocytes and embryos. The second is that in some experiments we will use 'transgenic' mice carrying a genetic mutation. In *all* cases the mice to be employed carry a mutation that makes an experiment possible that would otherwise not been feasible. For example, some mice carry a 'floxed' allele, allowing the function of a particular gene of interest to be examined. Some mice carry a GFP-transgene, allowing a particular protein to be observed using a specialised microscope. All of the transgenic mice to be employed experience normal health, and experience no suffering. All procedures are of mild severity, and no adverse effects are expected. Mice will be sacrificed by cervical dislocation in order to harvest cells.
Application of the 3Rs	
1. Replacement State why you need to use	Our work is in a highly specialised cell type — the oocyte and embryo. Eggs and embryos can only be

animals and why you cannot use non-animal alternatives	obtained as primary cells from laboratory animals, and there are no other cell type (such as a ‘cell line’) that could be used instead.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Hormones are administered to increase the number of eggs retrieved per mouse. Mouse strains selected on the basis of reliably high yields of eggs. Thus the number of animals used is kept to a minimum health.
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.	Mouse is the optimal model for studying mammalian oocyte and embryo development, as mouse development is well characterised, and because of the availability of transgenic mice that allow specific investigations to be carried out. No procedures are to be employed that would cause suffering or distress to animals. All mouse lines to be used experience normal health. Mice are monitored carefully to identify any deviations from normal health. Mice will be sacrificed by cervical dislocation, which causes the minimal possible distress to the animals.

PROJECT 21	**Biology of normal and leukaemic cells**	
Key Words (max. 5 words)	Leukaemia, haematopoiesis, therapeutic target, disease modelling.	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	By manipulating leukaemia genes in mice, we aim to produce the most relevant models for the disease that will allow us to validate hypotheses derived from our own and other's studies on primary human samples, tissue culture studies and epidemiology. The principle product of this endeavour is establishment of a sound, biological rationale for explaining underlying transformation mechanisms and development of more effective anti-cancer drugs for human leukaemia.	
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)?	Human leukaemia is a biologically and clinically diverse disease, and despite extraordinary advances in treatment over the past several decades, especially for childhood acute lymphoid leukaemia, outcome remains poor for some subtypes and long-term therapy related side effects are frequently associated with the treatment. Thus there is an urgent need to have better understanding of the disease and design more effective and less toxic therapies. The potential	

	benefits of this project are to understand how and why leukaemia develops, and to develop new cancer therapeutic drugs that are more effective at killing leukaemia cells and less toxic than current drugs.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. Approximate number of mice will be less than 12000 per year.
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?	Possible adverse effects include loss of condition, weight loss and effects on specific organs, e.g. bone marrow, spleen or/and liver. We set clear standards and guidelines for the performance of each procedure and monitoring the health status of the animals. Experiments will be stopped before distress or discomfort is apparent, using predefined limits of tumour size or adverse effect symptoms. Every effort will be made to minimise adverse effects by strict monitoring procedures and mice being humanely killed when endpoints are reached. However it is anticipated that on rare occasions severe adverse effects will occur and mice may die, this is because some leukemic cells are more aggressive in action than others and onset of adverse effects can occur within a short time frame. For this reason the severity limit has been set at severe for 3 protocols. The animals will be culled by schedule I method at the end of the protocol.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Prior to any evaluation in animals, a large amount of work is carried out using both biochemical approaches and testing in human cancer cells in tissue culture. Despite the optimal use of tissue culture systems, it is absolutely essential that part of the evaluation and development process is carried out in the intact animal, and indeed this is a gold standard for disease modelling and a requirement of regulatory bodies worldwide for drug development. This is because *1)* no in vitro model can recapitulate normal or malignant blood cell formation and 2) drugs have to be taken up into the body and be specific for cancerous but not normal cells. Only animal models provide the means to properly study the mechanisms

	and evaluate how new drugs will behave before using them directly on patients
2. Reduction Explain how you will assure the use of minimum numbers of animals	The outcome of the various procedures is reviewed regularly in order to consider the potential for reduction in animal numbers and the refinement or replacement of techniques. Any lessons learned from this review are disseminated to the research community. The numbers of mice are minimised according to the type of procedure, striking the optimal balance between reducing animal usage and obtaining robust and statistically significant results that will provide valid conclusions. This is extremely important since some of the results will form the basis for taking a drug forward for testing in patients.
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.	Mice were chosen for the project because *1)* it is the lowest vertebrate that shares similar genetics and biology as humans; 2) most of the cancer pathways are conserved between human and mouse; 3) procedure for manipulating mice for successful modelling is well established; and 4)the vast majority of drug evaluation and development is carried out in mice, and has been proved effective. Mice are monitored closely for signs of ill health and experiments will be stopped before distress or discomfort is apparent, using predefined limits of tumour size or adverse effect symptoms.

PROJECT 22	**Eye defects, development and repair**	
Key Words (max. 5 words)	Eye development, congenital, stem cell	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	Y	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to increase knowledge of the genetics of eye development, relevant to the causes of congenital eye defects and blindness, and to investigate ways of repairing diseased eye tissue. It will use mice as a model system for research as the mouse eye closely resembles the human eye and their genomes are very similar. Eye development begins in the fourth week of life in the human embryo and is completed after birth. It is a complex process that involves the coordinated maturation of several tissues. Disruption of development, or loss of proteins needed for sight, cause malformations and disease. In the UK about 10 in every 10,000 children are blind and in many cases the condition is incurable and the cause of the disease is unknown. Malformations, together with retinal disease, account for over half of the causes of blindness in children in the UK and are a significant cause worldwide.	

	Identification of the genetic changes underlying these conditions will provide better information for affected families and enables research to discover the biology of the condition and how it differs from normal. Knowledge of the genetic regulation of eye development presents new avenues for treatment and diagnosis. This may include repairing tissue by transplanting new cells into the eye or by promoting the diseased tissue to repair itself. In most of our investigations genetically modified mice, that model human eye diseases and/or that enable the study of particular genes and cells in the eye, will be bred and then used as a source of tissues and cells for analysis in the laboratory. We will use in vitro studies whenever possible, but these can only replace parts of whole animal investigations. Most animals will not used for live experimentation but will be humanely killed and used to provide tissue samples for analysis. When we do investigations using live mice, for example to transplant cells into the eye to repair diseased retina, the results will be relevant to clinical treatments for human eye disease, because of the similarities of the two systems. In such experiments, cells or pharmacological reagents will be injected into the eyes of animals under general anaesthetic. Several weeks after surgery, animals are humanely killed and the eyes removed for analysis. These studies will investigate new approaches to treat incurable causes of blindness.
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)?	This project will contribute to knowledge of the genetic basis of eye disease and to the future development of stem cell-based therapies for the treatment of blindness.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, <2000 per year
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?	
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mouse eye is very similar to the human eye. It develops in the same way and its structure, genetics and physiology is very similar. Mice carrying mutations in different genes develop eye defects and diseases that are like those caused by equivalent human genes. By studying mice with eye defects it is possible to gain insight into the disease process and to develop new approaches for treatment of blindness. It is impossible to directly study the human disease process because of the inaccessibility of human tissue for study.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project is designed to use minimal numbers of animals. The majority of animals will be used only for breeding to provide tissue for post mortem analysis.
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.	Study of mouse models of human eye disease is vital to advance understanding of the causes of human eye malformations and diseases causing blindness and for the development of new therapies. The benefit of mouse studies is the relatively straightforward extrapolation of results to humans, and therefore to clinical disease. Animal welfare will be upheld throughout.

PROJECT 23	**Improving light-activated therapies**	
Key Words (max. 5 words)	PDT, PCI, Photosensitiser, Chemotherapeutic, Laser	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	Y	Translational and applied research
	Y	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project focuses on understanding and optimising the parameters of light-activated therapies through different approaches to improve the efficacy of treatment and limit current adverse side-effects, with direct translation into the clinic.	
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 success of this project would allow us to come closer to optimising the clinical use of current drugs for treatment, in particular reducing either the amount of photosensitiser/chemotherapeutic administered or reducing the laser light exposure time. These findings can offer a reduction in treatment times and cutaneous photosensitivity whilst maintaining/improving the therapeutic effect, which will be extremely beneficial to the patient's quality of life.	

What species and approximate numbers of animals do you expect to use over what period of time?	It is estimated that an average of 2000 animals could be used annually in this project. This will only include rodents (800 mice, 800 rats and 400 hamsters). Rats and mice are mammals with the lowest neurophysiological sensitivity which are readily available. Hamsters have blood lipoprotein concentrations, important in drug biodistribution, highly similar to humans. These models also have organs of a suitable size for the proposed experiments.
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?	Procedures will involve systemic administration of a photosensitising agent, making the animals light-sensitive, and/or low doses of a chemotherapeutic agent, which should not be overtly toxic. The use of tumours grown in the breast, oesophagus, pancreas, cheek pouch, colon and subcutaneously in the flank for this project are intended to be the smallest tumour size necessary to reach a scientific endpoint e.g. reasonable to measure a volume of necrosis. Significant tumour burden and risk of metastases are not expected to occur. Targeted photosensitisers are to be used during light delivery therefore preventing damage to normal tissue. All surgical procedures carry some risk of excess bleeding and infection. *C. difficile* infection can cause inflammation of the intestine and acute diarrhoea however experience in this protocol has shown that the majority of animals are asymptomatic by the endpoint of the experiment. All treatments and procedures will be mild-moderate in severity and humane end points have been predetermined for each treatment group by Schedule I or non-schedule I methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Much preliminary PDT and PCI work using novel drugs will generally be tested *in vitro* for efficacy prior to *in vivo* studies and will continue to be carried out alongside animal studies (cell culture) to test the effectiveness and localisation of different photosensitisers, chemotherapeutics and other chemical agents. However, key aims of this program are to use optical pharmacokinetics to follow the distribution of photosensitising drugs around the body and to study how light-treated tissues heal. This can

	only be done in live animals. In addition, a significant component of PDT necrosis is thought to be caused through vascular effects. The consequence is that the study of PDT is dependent to a large degree on effects in living tissue which can only be assessed in an integrated 3D flow system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be kept to a minimum by observing *in vitro* affects and/or studying pharmacokinetic profiles *in vivo* of PDT/PCI agents prior to any treatment *in vivo,* in addition to ensuring all experiments are well designed, carried out effectively and colleagues collaborate wherever possible.
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.	Experiments will be carried out under general anaesthetic and pain relief administered whenever required to minimise suffering. Animals will be closely monitored and protected from bright lighting throughout. All procedures will have been ethically reviewed and all animals undergoing procedures will be monitored carefully by trained staff who work closely with a veterinary surgeon. This project will not involve any long-term growth delay studies.

PROJECT 24	**Coding sound in the normal and hearing impaired nervous system**	
Key Words (max. 5 words)	Hearing-impairment, cochlear implants, pitch, distortion	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Hearing aids can profoundly transform the lives of people with hearing impairments but in the UK alone about 6 million people who could benefit from a hearing aid do not use them. An important reason for this is that conventional hearing aids don't make it easy to distinguish meaningful signals such as speech from background noise. The combination of a fully functioning ear and the brain are fantastically good at this job and easily outperform the best computerized speech- recognition devices when even just a small amount of noise is present. Thus one major objective is to improve our understanding of how the brain detects signals in noise. Cochlear implants work extremely well but they generally fail to convey a sense of pitch which is an important property used in segregating sounds in noisy environments and conveying a sense of melody in	

	those with normal hearing. Thus a further objective aims to provide much needed information on why those with a cochlear implant do not have the perception of pitch. Finally we will provide new information of how the cochlea and auditory pathway responds to complex sounds which will help the production of new computer models of sound processing.
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)?	This project will improve our understanding of how the auditory brain encodes complex sounds in quiet and in adverse listening conditions. This information should enable the development of improved performance for those using hearing aids or cochlear implants. The same data will also provide much needed basic information on the role of distortion in complex sounds. This will inform the next generation of improved computer models of cochlear function.
What species and approximate numbers of animals do you expect to use over what period of time?	Guinea pig. Approximately 6 animals per month over 5 years. Total of 350 animals 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?	All experiments will be conducted under terminal anaesthesia and hence the harm factor is not applicable. All animals are killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We do not have satisfactory computational models of the responses of single neurons in the mammalian auditory pathway. As such the only way of obtaining this data is through electrophysiological recordings. These data will be used to provide further constraints on future modelling endeavours. Only *in-viva* experiments can examine the responses of single units to acoustic stimulation and the experiments would be impossible using *in-vitro* techniques.

2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed programme of research does not lend itself to the statistical calculation of the number of experiments needed in order to achieve significance. The experiments involve the search for neural codes of perceptual phenomena and, for instance, it is necessary to demonstrate that the responses of single neurons can be extended across cells differing in their best frequency and threshold. For example, it is typical to record the responses of well over fifty auditory-nerve fibres in a single experiment but the requirement that they all have best-frequencies **<** 3 kHz greatly reduces the yield. This yield is reduced still further when studying the extremely important, yet rare, high threshold auditory-nerve fibres. From extensive experience it is estimated that approximately twenty experiments will be run for projects on the auditory nerve. The typical number of experiments for studies carried out on central nuclei in the auditory pathway is again twenty The guinea pig is a suitable model for human hearing; cochlear gross structure is similar and recordings from the auditory nerve show many similarities with other mammalian species e.g. the cat, chinchilla, rabbit. We now have more information about how the guinea pig cochlea functions than for any other mammal. It is also worth pointing out that the use of mice or rats would be an unsuitable choice of animal for these experiments as they are unable to hear the low frequencies that are essential for the proposed experiments. API experiments in this project will be studied under terminal anaesthesia so the suffering is minimal. The experiments are classified as non-recovery on the severity rating scale.
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.	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.

PROJECT 25	**Injury and regeneration in the nervous system**	
Key Words (max. 5 words)	Multiple sclerosis, regeneration, stem cells	
Expected duration of the project (yrs)	5	
Purpose of the project as in	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	Y	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The nerve fibres of the brain are surrounded by an insulating material called myelin that protects the fibres and allows them to carry electrical impulses very rapidly. Myelin is made by cells called oligodendrocytes. These cells are lost in several neurological conditions including multiple sclerosis (MS) and several devastating inherited disorders. If myelin sheaths are not restored to nerve fibres then the nerve fibres themselves die, an irreversible change, which causes progressive clinical decline. Treatments are therefore needed to restore myelin to demyelinated nerve fibres and thereby protect them from degenerating. These will eventually involve drugs that make the brains own stem cells make oligodendrocytes restore the lost myelin more efficiently. However, in order to develop such urgently needed treatments it is necessary to know exactly how stem cells become new myelin forming oligodendrocytes. The objective of this project is to	

	contribute to this important translational research by addressing the following questions: Specifically, the key questions to be addressed are: 1) can we identify key regulatory networks that control CNS stem/progenitor cell maintenance and differentiation and, by manipulating these, influence CNS remyelination; 2) do adult stem/progenitor cells of different developmental origin or functional sub-types differ in their regenerative potential; 3) what are the physiological variables (primarily exercise and calorie restriction) that may exert an effect on the regenerative biology of CNS stem/precursor cells; 4) can lesion development and regeneration by differentiating stem/progenitor cells be visualised; 5) do the mechanical properties of normal and damaged CNS tissue influence regenerative processes and how could these be optimised for therapeutic benefit; and, 6) is paranodal demyelination a key component of axon dysfunction following chronic compression of the spinal cord?
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 programme of work will generate the knowledge base from which myelin regenerative drugs can be developed. Such drugs, which do not currently exist, will enable the progressive phase of multiple sclerosis to be treated, arresting or preventing disease progression, and, in conjunction which existing immunomodulatory treatments, from a comprehensive and effective way of treating this and other devastating diseases of myelin. We will also apply/develop imaging methods for visualising lesion development and subsequent remyelination in the living animal. The benefit of this is that it will significantly advance our understanding of how remyelination occurs, thereby facilitating new approaches by which it can be therapeutically enhanced.
What species and approximate numbers of animals do you expect to use over what period of time?	This project licence covers use of rats and mice. We expect to use approximately 9210 mice (including breeding colonies) and 2690 rats over a 5 year period.
In the context of what you propose to do to the animals,	For the animals covered by the application we do not expect any severe adverse effects in the vast majority

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	of them. However, some strains of genetically altered mice occasionally develop abnormal and potentially distressing signs of moderate severity, including brief fits and tremors. Some animals undergoing brain or spinal cord surgery will experience temporary functional disability (e.g. difficulty in walking, walking in circles, head tilts), which if persistent, will require the affected animal to be killed. Occasionally (<10%), when injections are made into the brain, there will be clinical signs that are classed as severe. Animals that reach humane endpoint such as persistent rolling (approximately 5%) will be killed. Ultimately, all animals kept under this PPL will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	CNS (brain and spinal cord) regeneration is a complex biological process involving many physiological and pathophysiological mechanisms. At present there are no non-animal systems that fully and accurately replicate CNS regeneration and that would therefore be suitable substitutes. For this reason animals are required to gather the level of knowledge required to meet the programmes objectives. Moreover, for many of the questions addressed it is necessary to use genetically altered mice in which the expression of specific genes potentially involved in CNS regeneration are changed. The genetic alterations required in our programme of work have only been generated in mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all experiments we aim to use the minimum number of animals compatible with obtaining significant results. Imaging methods will also be applied to observe lesion development and remyelination *in vivo,* which avoids killing animals at different time points. Experimental groups are chosen to contain age, sex and strain matched animals as far as is possible (on occasions where, for example, poorly breeding transgenic lines are used then groups may have mixed sexes in equal numbers). The group size is determined by the expected difference in

	outcome compared to controls. For experiments of expression of putative remyelination associated genes a minimum of four animals are required per period of time before the animals are killed. For functional studies the number of animals required per group is difficult to predict given that the likely effect is unknown. However, our previous experience with similar studies indicates that 6-8 animals per group are sufficient to give statistically meaningful data.
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.	Myelin only occurs in vertebrates and so non-vertebrate species are inappropriate for remyelinat ion studies. There are no demyelination/remyelination models that have been developed in birds, reptiles or amphibians although fish models are just beginning to be developed (as yet unpublished). However, despite similarities in fish and mammalian myelination there are important mechanistic differences. Furthermore, the overwhelming majority of studies on the biology of CNS regeneration have been undertaken using laboratory rodents, especially mice and rats. Thus, for this work to contribute to the main stream of translation-relevant research it is necessary to use mice and rats. These are also the mammals of the lowest neurophysiological sensitivity likely to produce satisfactory results. The lesion models proposed for inducing experimental demyelination have been chosen in part because of the minimal behavioural and locomotor deficits that they induce. Thus, lesions in the cerebellar peduncles and spinal white matter do not result in functional deficits discernable in cage-housed animals, i.e. the animals are able to move freely and perform normal physiological functions. In undertaking these procedures we are continually assessing how the procedures can be *refined* in order to minimise the discomfort that the animals may experience.

PROJECT 26	**Discovery ADMET studies for novel therapeutics**	
Key Words (max. 5 words)	ADMET, pharmacokinetics, discovery, DMPK, preclinical	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	N	Basic research
	N	Translational and applied research
	Y	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To provide a complete package of high quality, robust and incisive pre-clinica! pharmacokinetic data (what the body does to the drug) using ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicology) studies that will facilitate the rapid identification and selection of the best candidate drugs for further development and avoid any unnecessary and wasteful *in-vivo* tests being applied to inappropriate compounds. These approaches have been used in the past 20 years by most organisations involved in life-science R&D within UK, Europe and USA.	
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 likely benefit is greater success in clinical trials from more carefully selected compounds and a long-term reduction in the lead-time for a new therapy to reach patients. This will deliver a greater potential to save lives, alleviate suffering and reduce the incidence of adverse effects experienced with existing therapies.	

What species and approximate numbers of animals do you expect to use over what period of time?	For the duration of this licence, we will mainly be using rodents (mouse, rat, guinea pig, hamster, gerbil <6000 annually) but also non-rodent species (Rabbits, Pig and Sheep <150 annually)
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 expected adverse effects from the non-surgical and surgical models to be used under this licence are a) mild effects associated with the administration of novel compounds, such as hyperventilation and sedation that will not require further intervention or b) moderate severity adverse effects due to the animals undergoing surgical procedures, such as pain and discomfort requiring pain relief and fluid replacement. If any adverse effect which is more than transient, the animals will firstly be treated to alleviate the unwanted symptoms. In cases where the symptoms persist, after consultation with NACWO and Vet appropriate measures will be implemented that including termination by schedule 1 method. At the end of all protocols in this licence the animals will be killed humanely by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Currently, there is still a regulatory prerequisite by government authorities for new chemical entities (NCE's) to be tested *in-vivo* ADMET studies before first- in-man studies can be started. Also, current *in-vitro* systems lack the complexity of *in-vivo* models and cannot be used as a predictive tool to fully replace *in vivo* studies. Therefore, animals are still required to assess pre-clinical pharmacokinetics and are an essential part of drug discovery and development research.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In our aim to reduce the overall number of animals used for the duration of this licence, and as a good scientific principle, we always insist that there is a well established scientific rationale for undertaking any *invivo* study and that prior *in-vitro* data supports such studies. This careful selection of test compounds from *in-vitro* screening studies ensures that only those compounds with a

	positive profile for efficacy/potency, physic-chemical properties, metabolic profile and toxicity evaluation will be taken forward for use in regulated procedures in the species of the lowest acceptable order (i.e. rodents). As part of our commitment to minimise animal numbers, we will design our studies in such a manner that the maximum information can be obtained and where possible serial samples are taken from the same animal (i.e. by vascular cannulation) so as to negate the need for extra animals or future additional or repeat studies. The number of animals used for *in-vitro* assays in each experiment will be the minimum needed to provide sufficient cells/tissues. Additionally, we will use the minimum number of animals for the determination of pharmacokinetic parameters so as to achieve statistically relevant data. In some cases, it may be possible to limit animal numbers by reducing the size of control groups, sharing control groups or using control groups for analysing multiple outcomes.
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.	Non-surgical and surgical rodent models are the research species of choice in drug discovery and development due to their size and substantial amount of literature data already available. These models have been used and validated extensively, and have provided much of our knowledge to date in ADMET studies that are requested for use on this licence to generate the package of high quality, robust and incisive pre-clinical pharmacokinetic data that we aim to provide. However, on occasion other species (i.e. rabbits) may provide a much better correlation with humans, such as forming similar metabolites or exhibiting responses to a treatment are much more reflective of humans and in such cases we will ensure the most relevant species are always used. The choice of animals are also dictated by the governmental regulatory bodies as they demand that prior to first-in-man studies, pharmacokinetic data is provided in two species, rodent and a non-rodent, to ensure that the drug is suitable for human use. We will continuously monitor the literature to implement the latest animal husbandry and environmental enrichment legislation and practices. Furthermore, we will minimise animal suffering by using the most advanced technologies where possible, like non-invasive imaging, and by using appropriate anaesthetics, pain relief and infection controls.

PROJECT 27	**Development and plasticity of synapses and networks**	
Key Words (max. 5 words)	Nerve cell, synapse, development, energy, intracellular transport	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	N	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Nerve cells signal to each other by releasing chemical neurotransmitters at special sites called synapses. The scientific unknowns or scientific/clinical Nerve cells signal to each other by releasing chemical needs being addressed) neurotransmitters at special sites called synapses. The neurotransmitters act on receptors proteins to activate or inhibit the cell. A key goal is to better understand the currently poorly understood mechanisms that regulate the levels of the neurotransmitters and neurotransmitter receptors at the synapse, to regulate nerve cell communication. These mechanisms are also often disrupted in neurological disease. Altered receptor and transporter distribution and function is implicated in many neurological and neuropsychiatric diseases including epilepsy, stroke, Huntington's disease, anxiety, drug addiction, depression, autism and schizophrenia. Our aim is to better understand how synapse function is	

	modified by neurotransmitter receptor and neurotransmitter transporter trafficking which is crucial for understanding how the brain works, and may also lead to the identification of therapeutic interventions in a wide range of diseases. For neurons to continue to function they also need a constant energy supply, which is generated by the power-houses of the cell called mitochondria. Maintaining the correct function and distribution of mitochondria in large and complex cells like neurons (for example a motor neuron axon can be up to a meter long) is critical for brain function and has emerged as an important regulator of correct animal and brain development and physiology. Currently however the mechanisms that regulate mitochondrial dynamics are poorly understood. A key goal is to determine how neurons and glial cells regulate the position and function of the energy producing m itochondria. Defective m itochondrial trafficking and function is also implicated in many neurological and neurodegenerative diseases (like Alzheimer's disease and Parkinson's disease) and also causes cellular dysfunction in diseases like stroke, cardiac arrest and spinal cord injury. Studying the mechanisms that underlie these regulatory processes will allow us to understand better how the brain works under healthy conditions, and how dysregulation of these processes alters nerve cell function in disease.
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 main aim of this research project is to generate new scientific knowledge about receptor and organelfar trafficking and function and the role this plays in brain development, connectivity and function. Understanding how the brain forms connections (between neurons or between neurons and glial cells), maintains these connections, and regulates their strength, in the healthy brain are valuable in their own right and is also essential for understanding neurological disorders, such as epilepsy, Huntington's disease, Parkinson's disease, Alzheimer's disease, schizophrenia and autism, which exhibit disruptions in and/or abnormalities of neuronal development and signalling. The knowledge gained of the membrane trafficking properties of receptors, transporters and organelles in neurons, glia, and other cell types, and

	of the protein machinery and signalling mechanisms that regulate these processes, will substantially advance our understanding of the fundamental mechanisms by which nerve cells develop and communicate, and by which the brain functions. This knowledge will not only inform us regarding important mechanisms of animal and brain development and function but will also help to provide a basis for the development of therapeutic strategies, when these processes are disrupted in pathology.
What species and approximate numbers of animals do you expect to use over what period of time?	~ 1200 mice/year ~ 80 rats/year
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?	In some experiments we will use 'transgenic' mice carrying a genetic mutation. In all cases the mice to be employed carry a mutation that makes an experiment possible that would otherwise not been feasible. For example, some mice carry a 'floxed' allele, allowing the function of a particular gene of interest to be examined. Some mice carry a fluorescenttransgene, allowing a particular protein or cell type to be observed using a specialised microscope. No adverse effects are expected from the genetic manipulations. Some procedures of moderate severity will be performed, but appropriate anaesthesia and analgesia should ensure that animals do not experience lasting distress of pain. All mice and rats will be sacrificed or humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Live brain tissue and intact animals are essential for studying brain development and the properties of nerve cells and their connections. Moreover because some of the work to be carried out will study interactions between different cell types, it can only be done on tissue from intact animals.
2. Reduction Explain how you will assure	Each experiment is designed, as far as possible, to include its own control, reducing variability, increasing statistical sensitivity and thus minimizing the number

the use of minimum numbers of animals	of animals required to reach statistical significance. By using transgenic technology to make cells of a particular type and / or proteins within the cells fluoresce a particular colour, we can also reduce the number of animals 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.	Rodents have been chosen for this work as the lowest species which mimic the human nervous system well enough for our work to be relevant to human disease. Mice are essential because the experiments require the use of the latest mouse transgenic technologies to identify cell subtypes and to allow the knock-in and knock-out of genes. Mice are monitored carefully to identify any deviations from normal health. Mice will be sacrificed by a schedule 1 method, which causes the minimal possible distress to the animals or under anaesthesia.

PROJECT 28	**Toxicity Testing II**	
Key Words (max. 5 words)	Toxicology, rodent, medicine, appearance, histology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	N	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To carry our early safety testing of new medicines being developed for treatment of human diseases	
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)?	Before new medicines can be given to people they must be tested for potentially harmful effects (this is know as toxicity) as part of the evaluation of their advanced or humans or overall safety. Animals need to be used for these tests as this gives a much better idea of what might happen in humans than if the test were carried out using cells or other non-animal experiments. The toxicity testing of new medicines in animals is required by law, and must be performed before any human (clinical) studies can be undertaken. These experiments help to provide vital information to the scientists and allow them to run appropriate safe human studies, the next stage in the development of new medicines.	

	The purpose of this license will be to provide information of sufficient quality on potential new medicines that can be used to decide which of them are the least likely to show any adverse effects in further regulatory toxicology studies without the need for further experiments. Animals will usually be given the medicines by routes similar to those used in humans, by mouth (orally) into the lungs (inhalation), by injection or by a slow release route. The animals are observed regularly to look for a change in appearance and behaviour which may be an early sign of toxicity known as adverse effects. Weighing the animals daily identifies any changes in body weight as a loss in body weight is often an early sign of toxic effects in animals. Blood sampling from a vein to look for any changes in the blood chemistry is another good way of identifying toxicity. All these measurements and observations are used by doctors when monitoring patient's health. At the end of the study the animals are humanely killed and samples of various organs taken. These are examined under a microscope to see if the medicine has caused changes in the organs that would prevent administration to humans. This microscopic examination of tissue samples is similar to that used by doctors when studying biopsy samples taken from patients in hospital.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 1000 Rats 5000 Guinea pigs 1000 Over the 5 year period of this licence
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	As we are investigating the toxicity of the potential new medicines most studies conducted under this licence will induce some adverse effects in some of the animals being treated. Typical adverse effects include a reduction in body weight and/or reduced

happen to the animals at the end?	eating. Other effects may include changes in appearance, for example ruffled fur or changes in behaviour, for example the animals may become subdued. The larger proportion of animals used in these studies will, however, not experience any noticeable adverse effects this includes genetically altered animals which may have an inherent phenotype which is not expected to worsen as a result of being exposed to potential new medicines. In a very few studies devices that allow the slow release of the new medicine may be surgically implanted under the skin under a general anaesthetic. In addition small cannula may be surgically implanted into the tails of animals to help when taking blood samples. For the vast majority of animals the severity level will be mild. It is a requirement, however, that in some studies the new medicine must be given at a dose that does produce some adverse effects but these would only cause the animal a moderate level of distress. At the end of the study the animals will be humanely killed. After the animals are killed samples of body tissue are sent to laboratories for close examination to give more information about the effects of the potential new medicines.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is a point in the development of new medicines when using cells alone or other non- animal experiments cannot reproduce what happens in the whole human body. Using isolated cells, cultured cells or tissue samples can mimic some aspects of the disease, It is extremely difficult, however, to do non-animal experiments that are able to predict how a potential new medicine will be distributed around a body and if it will have a specific adverse effect on certain organs of the body. To fully understand these different interactions/effects animals have to be used. We will always ensure that the right amount of non-animal testing has been undertaken before running a study. Also we will ensure that the studies we plan to run have not been run previously elsewhere.
2. Reduction	By using our knowledge on experimental design and consulting other experts, each experiment will use the

Explain how you will assure the use of minimum numbers of animals	minimum number of animals required to ensure that the results obtained are reliable and allow decisions to be made on the development of the potential new medicine. How the studies are run and the results from them will be continuously reviewed to see if fewer animals can be used and still produce results that will help in the development of new medicines. This licence is to look at potential new medicines that have a good chance of being used in patients. As such the number of new medicines being investigated and therefore the number of studies carried out is predicted to be low.
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 also use guinea pigs in some of our experiments because their airways are generally more similar to human airways than the airways of other rodents. We have developed Special Welfare Assessment Sheets (WAS) which allow us to identify the most humane point at which to stop an experiment. These sheets will allowed us to identify relatively minor reactions to a potential new medicine which we know will get worst over time and stop an experiment before this happens. Painkillers will be given to the animals after taking advice from a veterinary surgeon. Mice and rats are the best animals to use in this kind of toxicity study. Their mammalian bodies are incredibly similar to those of humans in many respects and provide a good way of predicting how a medicine will react inside the human body. A great deal is already known about the effects of medicines on mice and rats and this information is used when new medicines are being developed.

PROJECT 29	**FMOs: exogenous and endogenous metabolism**	
Key Words (max. 5 words)	Trimethylaminuria; drug therapy; metabolism	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	Y	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) (Part 1)	This project involves mouse models to help identify a therapeutic for use in the treatment of the genetic disorder trimethylaminuria (TMAU). This disorder arises because individuals have mutations in the gene called FMO3 that prevent the conversion, in the liver, of trimethylamine to trimethylamine N- oxide. Trimethylamine is produced, by the bacteria in the gut from normal dietary foods containing a substance called choline. Trimethylamine is the chemical that gives rotting fish its characteristic smell, while the trimethylamine N-oxide does not smell. Thus, people who cannot convert trimethylamine to the non-odorous product excrete large amounts of trimethylamine in their breath, urine and sweat. This disorder severely influences the quality of life. The male mouse, when it reaches adulthood, stops producing FMO3 protein and begins to excrete large amounts of trimethylamine in urine. Thus the male mouse provides an excellent model to test a therapeutic for its ability to reduce trimethylamine in the urine. Mice will be treated with the therapeutic by different routes and	

	different doses and the concentration of trimethylamine and its N-oxide in urine will be determined. A successful outcome will be judged by the reduction of trimethylamine and an increase in trimethylamine N-oxide. The bioavailability and the distribution of the therapeutic will also be examined by the analysis of blood and tissue samples. It is important to test a therapeutic also in female mice. Female mice, unlike male mice, continue to produce the FMO3 protein in adulthood. By feeding female mice with choline we can mimic the condition of TMAU. This is because increased choline in the diet will lead to an increase in the production of trimethylamine. Thus this choline-fed model will test the benefit and influences of the therapeutic in females.
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) (Part 2)	FMO3, together with other members of the FMO family of proteins, are important enzymes that enable us to convert therapeutic drugs to chemicals that can be easily excreted. Drugs are prescribed in a certain concentration so that there is sufficient of it to reach its site of action. The body must however, be able to remove any excess drug and so prevent drug overdose or what is called an adverse drug reaction. We will use mouse models in which the genes for different FMO proteins have been inactivated or deleted to examine the consequences of the absence of a FMO protein on drug metabolism and drug clearance. In this way we will be informed of how a particular FMO protein is involved in the metabolism of a drug. Mouse models in which FMO genes have been inactivated or deleted show that these proteins also play a role in the metabolism of endogenous chemicals. We will investigate the processes in which FMO proteins influence metabolism. We will also identify changes in normal metabolism as a consequence of drug administration. In some experiments we will use cultures of hepatocytes (liver cells isolated from animals and grown in the laboratory) to investigate drug and endogenous metabolism.
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 key benefits are a potential therapeutic for treatment of trimethylaminuria in humans. This will help alleviate the symptoms and thus improve quality of life. In addition, our studies will inform on better efficacy for drug therapy through an avoidance of adverse drug reactions and an understanding of how drugs influence endogenous metabolism.

What species and approximate numbers of animals do you expect to use over what period of time?	Mice that are genetically altered for drug metabolising enzymes or wild-type mice (450). Rats (10).
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?	Protocols have mild status, except for the delivery of the therapeutic for trimethylaminuria (moderate). Animals may experience slight discomfort on injection. Adverse effects are expected to be mild and minimised through the use of local anaesthesia when blood samples are taken. Tissues will be harvested or cells will be obtained for culture. In either case the animals will be killed by a Schedule I procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Preclinical trials require the establishment of efficacy in animals before therapy and dose can be predicted for human use. Studies on the interaction of drugs and endogenous metabolism require studies on multiple tissues, blood, urine and feces.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal use is determined by pilot studies and statistical analyses. Multiple analyses will be carried out on tissues, blood, urine and feces from individual animals. Some experiments will be carried out on cells cultured from animal tissues. This increases the number of treatments possible and reduces the number of animals.
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.	For the testing of a therapeutic for trimethylaminuria male mice will be used — as they are a natural knockout for the FMO3 gene (which in humans is the gene affected by trimethylaminuria). Drug and endogenous metabolism studies will use genetically altered mice in which genes for enzymes that metabolise drugs have been altered to inactivate the gene.

PROJECT 30	**Information processing in mammalian brain circuits**	
Key Words (max. 5 words)	neuron dendrite synapse sensory rodent	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	N	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Neurons are the basic cellular units of the brain, and are connected via synapses to form neural networks. One of the central questions in neuroscience is how particular tasks, or “computations”, are implemented by neural networks to generate behaviour, and how patterns of activity are stored during learning.	
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 results of this project will extend our basic knowledge of the fundamental processes underlying information processing and memory storage in mammalian central neurons. This is essential if we are to understand how neurons communicate with each other and how information is processed and stored by networks of neurons in the intact brain. In the long term the results of these experiments, and the techniques we have developed, will provide new approaches of potential valuable for understanding and treating disorders of brain function such as occur in stroke, ischemia, hereditary ataxias, epilepsy and	

	dementia.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (11,875) Rats (1300) 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?	Most animals will undergo only mild or unclassified procedures. Less than 30% of animals will undergo procedures that are expected to be moderate as they may result in some post-operative pain and initial stress on head-fixation. Pain will be limited by use of analgesics. Stress will be limited by gradual introduction of head restraint and provision of ample water, sugar- water or food rewards. Animals that are head-fixed but supported on a floating ball system are free to run voluntarily and do not show signs of stress from the head restraint. Where possible, this system will be used. Animals will be killed by schedule 1 at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are needed in order to study intact brain circuits and their involvement in encoding sensory responses and driving behaviour.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use advanced statistical tests (e.g. KolmogorovSmirnov, Wilcoxon matched-pair tests) in order to use the minimal number of data points to provide statistically significant results, and for multigroup comparisons will use the appropriate tests (e.g. ANOVA).
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	Rodents are probabiy the lowest species for which direct comparison can be made with the structure and functioning of the human brain. Our primary experimental model system is the mouse, which is currently the species of choice in most areas of biomedical research, as it allows the use of powerful techniques such as the generation of transgenic animals. This has enabled the selective expression of specific molecules, such as fluorescent proteins, in

(harms) to the animals.	identified populations of neurons to facilitate target-directed recordings instead of random target selection. This is a refinement of our methods and it reduces the number of animals used, Head restraint systems have been optimised over the course of the last PPL and the introduction of the floating ball treadmill has substantially reduced stress. We will continue optimising this system to improve stability of the recordings and thus enable more inclusive use of the floating ball.

PROJECT 31	**External Fish Parasites and Therapeutics**	
Key Words (max. 5 words)	Aquaculture, parasite, fish	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	N	Basic research
	Y	Translational and applied research
	Y	Regulatory use and routine production
	Y	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Summarise your project	The effects of therapeutants on external fish parasites	
Objectives	Fish can suffer from naturally occurring outbreaks of parasite infections that cause poor welfare by adverse effects such as itchiness, loss of scales, weight loss, poor ability to control their salt/water balance and death if the parasite load becomes high. There is developing resistance within the parasite population to currently licensed products, meaning that treatments are often not fully effective or are not effective at all in killing the parasites. This project seeks to investigate treatments that provide new or improved control methods.	
Outline the general project plan	Parasites will be exposed to potential therapeutants to find those that give promising results and to identify effective dose and durations. Providing effective doses and durations do not adversely affect the fish, the external parasites will be	

	checked before and after their exposure to the therapeutants. Testing the efficacy of existing therapeutics will be done at dose and durations informed by that used on farms. Fish will be monitored throughout the whole process. If naturally infected fish are not available from fish farms, small numbers will be infected by exposing them to parasite infective stages and these fish will be allowed to develop mild disease, mimicking that seen naturally.
Predicted harms	Fish will be exposed to potential treatments for parasites. The treatment will usually be put into the water, but may also be put into food or injected when the fish is asleep under an anaesthetic. The fish will either have the parasites on them because they have been exposed to the parasite in the nature environment or the parasite may be introduced by exposure in water if it is not possible for us to source naturally infected fish.
Predicted benefits	Parasite infections on fish farms can negatively affect fish welfare and can result in underperformance and incur significant costs. The project will primarily benefit the fish stocks by improving the effectiveness or availability of treatments, but also the food producers by improving growth rates and fish quality and also reduce parasite loading of the environment, which will in turn protects wild stocks of fish. The fish will be monitored over a few days to a few weeks to see whether the treatment works. In order to check the number of parasites it may be necessary to give the fish one or more brief anaesthetics to allow parasite numbers to be assessed without stressing the fish. In some studies we may need to increase how many fish are kept together or change the rate the water flows at through the tanks to mimic situations that occur on fish farms, if we think this is important in whether or not a treatment will work.
Estimate the numbers of animals of each species to be used	It is estimated that no more than 500 Atlantic salmon will be used in this project. This species dominates the UK aquaculture sector and would be the primary

	benefactor of the anti-parasite medicines. The minimum number of salmon would be ensured by careful design of experiments and by screening the effect of therapeutants on the parasites alone before incorporating salmon in the trials to test the most promising treatments.
Demonstration of compliance with the 3Rs	The only current model for testing effects of therapeutants on parasite and host is through exposure to the therapeutant. For those parasites capable of living independent from the host, testing of the parasite in lab studies will help identify effective dose and exposure duration prior to testing on the infected host.
Explain why the protocols and the way they are carried out should involve the least suffering	Infection rates will be kept to a level where only mild irritation should be experienced. Fish will be closely monitored before, during and after treatments and any animal showing signs of obvious poor health will be treated or humanely killed.

PROJECT 32	**Breeding and maintenance of genetically altered mice for central nervous system regeneration research**	
Key Words (max. 5 words)	Multiple sclerosis, regeneration, stem cell	
Expected duration of the project (yrs)	12	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	Y	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The nerve fibres of the brain are surrounded by an insulting material called myelin that protects the fibres and allows them to carry electrical impulses very rapidly. Myelin is made by cells called oligodendrocytes. These cells are lost in several neurological including multiple sclerosis (MS) and several devastating inherited disorders. If myelin sheaths are not restored to nerve fibres then the nerve fibres themselves die, an irreversible change, which causes progressive clinical decline. Treatments are therefore needed to restore myelin to demyelinated nerve fibres and thereby protect them from degenerating. These will eventually involve drugs that make the brains own stem cells make oligodendrocytes more efficiently. However, in order to develop such urgently needed treatments it is necessary to know exactly how stem cells become tie	

	myelin forming oligodendrocytes. This is the primary objective of our programme of work. However in order to achieve this it is necessary to breed and maintain your GAAs while a new PPL is written.
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 programme of work will generate the knowledge base from which myelin regenerative drugs can be developed. Such drugs, which do not currently exist, will enable the progressive phase of multiple sclerosis to be treated, arresting or preventing disease progression, and, in conjunction which existing immunomodulatory treatments, which from a comprehensive and effective way of treating this and other devastating disease of myelin. The benefit of this licence is to enable us to breed and maintain valuable GAAs for our main programme of work, which might otherwise be lost.
What species and approximate numbers of animals do you expect to use over what period of time?	This project licence covers the breeding and maintenance of genetically altered mice. We expect to use approximately 600 mice over a 12 month period.
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?	For the animals covered by the application we do not expect any adverse effects in the majority of them. However, some strains of genetically altered mice occasionally develop abnormal and potentially distressing signs of moderate severity, including brief fits and tremors. Some of the mice bred under this licence will subsequently be used in studies covered in a larger PPL currently being written. Ultimately, all animals kept under this PPL will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	CNS (brain and spinal cord) regeneration is a complex biological process involving many physiological and pathophysiological mechanisms. At present there are no non-animal systems that fully and accurately replicate CNS regeneration and that would therefore be suitable substitutes. For this reason animals are required to gather the level of knowledge required to meet the programmes objectives. Moreover, for many of the questions

	addressed it is necessary to use genetically altered mice in which the expression of specific gene potentially involved in CNS regeneration are changed. The genetic alterations required in our programme of work have only been generated in mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will maintain the breeding colonies at the minimum level necessary.
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 genetic alterations required in our programme of work have only been generated in mice. These mice will be eventually be used in procedures covered by another PPL application. In the experiments proposed in the broader programme small focal areas of damage will be induced that are sufficient to address the questions posed by the experiment but are small enough to cause minimal or undetectable function changes in the experimental animal. In this way we are able to refine our experiments such that they are consistent with the objectives but cause the minimum disturbance to the animals involved. Some of the GAAs in this application develop moderate signs. For breeding and maintenance these will be minimised by maintaining animals as heterozygotes to reduce or prevent adverse phenotypic expression.

PROJECT 33

Assessing the feasibility of using an animal model for in vivo taste assessment of pharmaceutical compounds and formulations

KEYWORDS

taste assessment, lickometer, rodents, APIs, formulations

• Summarise your project (1-2 sentences)

In this project we would like to set up, explore and validate the rodent brief-access taste aversion model to assess palatability of medicines at the early phase of dosage form development including for paediatrics.

• 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.

Taste is crucial for patient acceptability and compliance, especially for children. However, most of the active pharmaceutical ingredients (APIs) are bitter therefore taste masking has become essential. Nevertheless, assessing the taste by human panels can only be done during advanced drug development when toxicology data of the drug are sufficient. Discovering the unpleasant taste of a formulation when the drug development is well advanced can lead to increasing risk of failure and stop of the drug development program. Thus, there is an urgent need to develop or optimise a taste assessment method in the very early drug development phase.

• Outline the general project plan.

The model will first of all be performed with adult rodents with established tastants. In parallel to the assessment of the animal taste model, the taste intensity of the same compounds will be scored by adult human volunteers as well as measured with taste sensors (e-tongue) in order to compare the results. The same procedure will be then repeated with post-weaning, adolescents, young adults and elderly rodents as taste perception can vary with ageing.

• 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.

A lickometer which is an apparatus that enables to electronically record the licks of water- deprived animals during very brief taste stimulus exposure will be used. Decreases and increases in lick rate, relative to water, will be taken as a measure of oral aversiveness and appetitiveness respectively. The

rodents will be exposed to the solutions such that “no observable effect levels” are reached. Moreover, when animals are placed in an intermittent water-deprivation schedule (maximum 5 days), the weight of each rodent will be checked every day and if the weight drops below 85% of their free-feeding weight, they will receive adequate hydration to recover their normal weight. • Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.

With this “animal taste model” taste could be evaluated at the beginning of the pharmaceutical development. Therefore, it would be possible to screen and select the most palatable drug candidates enabling to be more effectively taste-masked in future development stages which is at the moment nearly impossible due to lack of adequate methodology. Moreover, in our project the tool will enable selection of the most appropriate excipients for taste-masking by enabling assessment of the early formulations developed.

Last but not least, there is little knowledge and evidence of differences of taste perception in paediatric and elderly patients. This method will therefore be the first to assess the taste of formulations in young and old animals and provide a juvenile taste animal model if earlier hypothesis demonstrate it.

Leading this project is the essential need to characterise taste early in medicine development, due to the importance of meeting the patient needs for successful therapy.

• 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.

Rodents are most often used in taste research. In particular, rats and mice have been widely used for taste perception studies for many reasons: their high drinking capability; their taste similarity to humans and the large corresponding literature available on the comparison of humans and rodents; and their short lifespan. Therefore, all our protocols and equipments will be optimised for these species.

In order to reduce the number of animals that will be used in the study, we are planning to only utilise the animals required to carry out acceptable statistics analysis (up to 300 rodents). A statistician will be consulted to have further advice on the number of animals needed to have reliable results. Moreover, later on, as we will conduct studies across nearly all the lifespan of the animal, when our study will start with juvenile post-weaning rodents, the same animal will be used when the influence of age on taste will be inspected on adolescent, young adult, adult and elderly rodent.

• 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.

It is envisaged that the animal taste model will be a screening tool for taste assessment at the very early phases of drug development. At this stage of formulation development, a lack of sufficient toxicology data prevents the use of humans to assess the taste of new molecules or formulations, It is therefore proposed that rodents act as a surrogate in vivo tool.

Another alternative we considered and that we will use in addition to our animal model is an analytical instrument made with lipidic or polymeric sensors known as the e-tongue. This in vitro tool is a novel instrument which has yet to be fully correlated to the human taste; therefore it cannot be used as a single taste assessment tool.

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

No invasive procedure will be undertaken. The rodents will be presented with tastants solutions at non-toxic level.

The rodent brief-access taste aversion model has been shown to be completely safe for the animals with no impact on their health and behaviour.

As a safety and welfare measure, when the animals are placed on an intermittent waterdeprivation schedule to make them drink, the weight of each rodent will be checked every day and if the weight drops below 85% of their free-feeding weight, they will receive adequate hydration to recover their normal weight. However this schedule has been used extensively safely in the literature.

PROJECT 34	**Safety and efficacy of veterinary medicines**	
Key Words (max. 5 words)	Veterinary medicines safety efficacy testing	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	N	Basic research
	Y	Translational and applied research
	Y	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of the project are to generate cata on the safety and efficacy of novel veterinary medicines, which can be used by the pharmaceutical company to support their claim for a new/modified marketing authorisation for the product.	
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 potential benefit of this project is the development of new veterinary medicines, which could be used to prevent or treat disease in farm animal species, thereby improving the welfare of these animals.	
What species and approximate numbers of animals do you expect to use over what period of time?	The project expects to use approximately 300 dairy cattle over a five year period.	
In the context of what you propose to do to the animals,	The procedures the animals are likely to encounter are mild in severity, and often conducted as acts of	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	veterinary surgery (e.g. collection of blood samples), but as they are for the purpose of research, they require authorisation under ASPA.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune systems of mammals are complex, and are influenced by a number of ancillary systems, such as the digestive system and the circulatory system. At the 'present time, it is not possible to model the response of an animal to a veterinary medicine, which therefore necessitates the use of animals in studies to determine their safety and efficacy. In addition, the regulatory requirements for a Marketing Authorisation for a veterinary medicine in the EU require that safety and efficacy are demonstrated in the target species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculations are conducted by a statistician, to determine the optimum number of animals required to demonstrate efficacy.
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 study will be conducted in the target species for the veterinary medicine. The study sites are selected based on the presence or absence of a clinical condition/disease relevant to the medicine being evaluated. Animal suffering will be minimised by having an appropriate protocol for monitoring study animals, veterinary involvement and clearly defined end-points.